

NEUROSCIENCE IN MEDICINE

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THIRD EDITION

Edited by

P. MICHAEL CONN

Oregon National Primate Research Center

Oregon Health and Science University

Beaverton, OR, USA

Editor

Dr. P. Michael Conn
Oregon Health & Science University
Oregon National Primate Research Center
Div. Neuroscience
505 NW 185th Ave.
Beaverton OR 97006
USA
connm@ohsu.edu

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PREFACE

When the first edition of this book was prepared, we were midway into the “Decade of the Brain”—the 1990s. The passage of time has allowed the promise of this field to become the reality of progress. Research in neuroscience and remarkable technology have now made material contributions to human well-being.

As in the first two editions, the challenge remained to define the “core material” in a rapidly expanding field. I have continued to restrict peripheral areas (cell function and biosynthesis, for example) in order to focus on emerging and important areas that would not be found in a more generalist text.

The book has benefited by supportive contributors who were carefully selected because they are both excellent teachers and academic leaders. That they chose to contribute the time and energy needed for this project is a strong endorsement of their commitment to this discipline and to this project. A substantial number of the authors have been participating since the first edition.

I am pleased to continue the tradition of presenting “Clinical Correlations” for select chapters and that Dr. Greg Cooper has taken over primary responsibility for this task. Because of their popularity, Dr. Cooper has expanded the number of these.

Another feature of this edition is the addition of an Interactive Atlas, which is provided on a CD.

In late 2006, as preparations were being made for initiation of this revision, we learned of the loss of David V. Smith, Ph.D., who succumbed to a brain tumor at the age of 63. Dr. Smith was the Simon R. Bruesch Professor and Chair of the Department of Anatomy & Neurobiology and the Director of the Neuroscience Institute at the University of Tennessee Health Science Center (UTHSC) and a contributor to the earlier editions of this book. We miss his presence in the current revision.

Finally, I thank the staff at Springer for guiding me through this process.

*P. Michael Conn
Portland, Oregon*

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CONTRIBUTORS

- JOHN D. BOUGHTER JR., PhD • *Department of Anatomy & Neurobiology, University of Tennessee Health Sciences Center, Memphis, TN, USA*
- SONIA L. CARLSON WATSON, PhD • *Rochester, MN, USA*
- RAFAEL C. CARUSO, MD, PhD • *OGVFB, National Institutes of Health, Bethesda, MD, USA*
- RACHEL CASAS, BA • *Department of Psychology, University of Iowa Hospitals and Clinics, Iowa City, IA, USA*
- ROCHELLE S. COHEN, PhD • *Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL, USA*
- GREGORY COOPER, MD, PhD • *Sanders-Brown Center on Aging, The University of Kentucky, Lexington, KY, USA; Baptist Neurology Center, Lexington, KY, USA*
- GERALD EICHHORN, MD • *The Lexington Clinic, Lexington, KY, USA*
- MATTHEW ENNIS, PhD • *Department of Anatomy & Neurobiology, University of Tennessee Health Science Center, Memphis, TN, USA*
- EDMOND J. FITZGIBBON, MD • *National Eye Institute, National Institutes of Health, Bethesda, MD, USA*
- MARC E. FREEMAN, PhD • *Department of Biological Science, Florida State University, Tallahassee, FL, USA*
- HENRIQUE VON GERSDORFF, PhD • *Oregon Health & Science University, The Vollum Institute, Portland, OR, USA*
- A. TUCKER GLEASON, PhD • *Department of Otolaryngology–Head & Neck Surgery, University of Virginia, Charlottesville, VA, USA*
- CHARLES R. GOODLETT, PhD • *Department of Psychology and Program in Medical Neuroscience, Indiana University–Purdue University Indianapolis, Indianapolis, IN, USA*
- DAVID R. GRATTAN, PhD • *University of Otago Medical School, Department of Anatomy and Structural Biology, Dunedin, New Zealand*
- THOMAS VAN GROEN, PhD • *Department of Cell Biology, The University of Alabama at Birmingham, Birmingham, AL, USA*
- ROBERT D. GRUBBS, PhD • *Department of Pharmacology, School of Medicine, University of Washington, Seattle, WA, USA; Department of Pharmacology and Toxicology, Boonshoft School of Medicine, Wright State University, Dayton, OH, USA*
- G. JEAN HARRY, PhD • *Neurotoxicology Group, Laboratory of Neurobiology, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC, USA*
- CHARLES J. HECKMAN, PhD • *Department of Physiology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA*
- MARY M. HEINRICHER, PhD • *Department of Neurological Surgery, Oregon Health & Science University, Portland, OR, USA*
- J. FIELDING HEJTMANCIK, MD, PhD • *National Eye Institute, National Institutes of Health, Bethesda, MD, USA*
- LAWRENCE VER HOEF, MD • *Department of Cell Biology, The University of Alabama at Birmingham, Birmingham, AL, USA*
- THOMAS A. HOUPT, PhD • *Department of Biological Science, Florida State University, Tallahassee, FL, USA*
- RAJAN JAIN, MD • *Henry Ford Hospital, Department of Radiology, Detroit, MI, USA*
- CONRAD E. JOHANSON, PhD • *Program in Neurosurgery, Department of Clinical Neurosciences, Warren Alpert Medical School, Brown University, Providence, RI, USA*
- STEVEN J. ST. JOHN, PhD • *Department of Psychology, Rollins College, Winter Park, FL, USA*
- INGA KADISH, PhD • *Department of Cell Biology, The University of Alabama at Birmingham, Birmingham, AL, USA*
- MICHAEL A. LANE, PhD • *Department of Neuroscience, University of Florida College of Medicine and McKnight Brain Institute, Gainesville, FL, USA*
- PATRICIA LIMOUSIN, MD, PhD • *Unit of Functional Neurosurgery, Institute of Neurology, University College, London, United Kingdom*
- MICHAEL D. LUMPKIN, PhD • *Department of Physiology and Biophysics, Georgetown University Medical School, Washington, DC, USA*
- IRENE MARTINEZ-TORRES, MD • *Unit of Functional Neurosurgery, Institute of Neurology, University College, London, United Kingdom*
- ROBERT W. MCCARLEY, MD • *Professor and Chair, Department of Psychiatry, and Director, Neuroscience Laboratory, Harvard Medical School Department of Psychiatry, Associate Director*

- Mental Health Services, VA Boston Healthcare System, Brockton, MA, USA*
COLLEEN A. McCUNG, PhD • *UT Southwestern Medical Center, Department of Psychiatry and Center for Basic Neuroscience, Dallas, TX, USA*
- MICHAEL W. MILLER, PhD • Department of Neuroscience and Physiology, State University of New York–Upstate Medical University, Syracuse, NY, USA*
- ROBERT E. MRAK, MD, PhD • Department of Pathology, University of Toledo Health Sciences Campus, Toledo, OH, USA*
- MARION MURRAY, PhD • Drexel University College of Medicine, Department of Neurobiology and Anatomy, Philadelphia, PA, USA*
- DWIGHT M. NANCE, PhD • Susan Samueli Center for Integrative Medicine, University of California, Irvine, Orange, CA, USA*
- BRUCE W. NEWTON, PhD • Department of Neurobiology and Developmental Sciences, University of Arkansas for Medical Sciences, Little Rock, AR, USA*
- SURESH C. PATEL, MD • Henry Ford Hospital, Department of Radiology, Detroit, MI, USA*
- DONALD W. PFAFF, PhD • Professor of Neurobiology and Behavior, The Rockefeller University, New York, NY, USA*
- ADAM C. PUCHE, PhD • Department of Anatomy & Neurobiology, University of Maryland, Program in Neuroscience, Baltimore, MD, USA*
- PAUL J. REIER, PhD • Department of Neuroscience, University of Florida College of Medicine and McKnight Brain Institute, Gainesville, FL, USA*
- ROBERT RODNITZKY, MD • Department Head of Neurology, University of Iowa Hospitals and Clinics, Iowa City, IA, USA*
- SCOTT J. RUSSO, PhD • UT Southwestern Medical Center, Department of Psychiatry and Center for Basic Neuroscience, Dallas, TX, USA*
- WILLIAM Z. RYMER, MD, PhD • Rehabilitation Institute of Chicago, Chicago, IL, USA*
- MICHAEL T. SHIPLEY, PhD • Department of Anatomy & Neurobiology, University of Maryland, Program in Neuroscience, Baltimore, MD, USA*
- YOLAND SMITH, PhD • Yerkes National Primate Research Center and Department of Neurology, Emory University, Atlanta, GA, USA*
- STANKO S. STOJILKOVIC, PhD • Section on Cellular Signaling, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA*
- STEPHEN TISCH, MD, PhD • Unit of Functional Neurosurgery, Institute of Neurology, University College, London, United Kingdom*
- HAROLD H. TRAURIG, PhD • Department of Anatomy and Neurobiology, University of Kentucky College of Medicine, Lexington, KY, USA*
- CHRISTINA T. TENG, PhD • Gene Regulation Section, Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC, USA*
- DANIEL TRANEL, PhD • Department of Neurology, University of Iowa Hospitals and Clinics, Iowa City, IA, USA*
- BRENT A. VOGT, PhD • Department of Neuroscience and Physiology, State University of New York–Upstate Medical University, Syracuse, NY, USA*
- SIMONE WAGNER, MD • Department of Neurology, University of Heidelberg, Heidelberg, Germany*
- J. MICHAEL WYSS, PhD • Department of Cell Biology, The University of Alabama at Birmingham, Birmingham, AL, USA*
- TOM C.T. YIN, PhD • Department of Physiology, Neuroscience Training Program, University of Wisconsin-Madison, Madison, WI, USA*

LIST OF COLOR PLATES

Color plates follow p. 378.

- Color Plate 1** An electron micrograph of a CNS synapse. This example of a synaptic bouton-type synapse is located in the “molecular layer” of rat cerebellum. A single *en passant* bouton of the parallel fibers synapses onto a single Purkinje cell spine. Note the multiple synaptic vesicles in the presynaptic bouton terminal. Several vesicles seem to be linked by thin filaments in the cytoplasm. On average, the vesicles have a diameter of about 40 nm. One synaptic vesicle is clearly docked to the presynaptic membrane. Note also the narrow synaptic cleft, which contains a “fuzzy” set of electron-dense material (this probably includes cell adhesion proteins that span the cleft). The opposing postsynaptic membrane in the postsynaptic spine has an electron-dense postsynaptic density (PSD), where glutamate receptors and modulatory proteins are located. A thin glial process wraps itself around the synaptic cleft and postsynaptic spine and also partially around the presynaptic bouton-type terminal. (Electron micrograph courtesy of Constantino Sotelo, Instituto de Neurociencias de Alicante, Spain).
- Color Plate 2** A schematic diagram showing the sequence of events that leads to Wallerian (anterograde) degeneration. The normal cytology of a peripheral nerve is shown as a point of reference (expanded inset **A**). After axonal injury, the proximal stumps retract from the site of injury forming distinctive “retraction bulbs” (expanded inset **B**). Meanwhile, the distal portion of injured axons degenerate, but all other elements of the peripheral nerve remain intact (expanded inset **C**). Thereafter, Schwann cells begin to proliferate, and blood-borne macrophages infiltrate the degenerating nerve stump and assist Schwann cells with phagocytosis of axonal and myelin debris (expanded inset **B**). Schwann cells then become arranged in columns known as the “bands of Bungar” within common basal laminae (expanded inset **C**). Such Schwann cell units provide a cellular pathway along which regenerating axons extend distal to the site of injury (*see also* Fig. 10).
- Color Plate 3** **(A)** A three-dimensional rendition of early axonal regeneration after peripheral-nerve damage. Growth cones (e.g., *boxed profile*) are seen extending into the lesion gap (*blue profiles* representing connective tissue elements), and some make contact with Schwann cells (*red profiles*) in the distal stump. (Drawing kindly provided by Dr. Susan E. Mackinnon, Washington University School of Medicine and Barnes-Jewish Hospital.) **(B)** An axonal growth cone of an embryonic chick sensory neuron is shown. The growth cone is doubly stained with an antibody against tubulin (*green*), which labels microtubules, and rhodamine-phalloidin to label actin filaments *red*. The bundle of axonal microtubules (*green*) splays apart in the growth cone, and individual microtubules extend forward to interact with actin filament bundles and networks. These interactions between actin filaments and microtubules are important in determining directions of axonal growth and branching (*see text*). A small axonal sprout has formed at the lower left margin of the growth cone. (Figure generously provided by Paul C. Letourneau, Ph.D., University of Minnesota.)

Cytology and Organization of Cell Types: Light and Electron Microscopy

Rochelle S. Cohen and Donald W. Pfaff

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- NEURONAL RESPONSE TO A CHANGING ENVIRONMENT
 - MECHANISMS OF NEURONAL FUNCTION
 - CYTOSKELETON DETERMINATION OF NEURONAL FORM
 - NEURONAL SYNAPSES
 - GLIAL CELLS
 - SELECTED READING
-

1. NEURONAL RESPONSE TO A CHANGING ENVIRONMENT

Although neurons are cells that conform to fundamental cellular and molecular principles, they are differentiated from other cells in ways that reflect their unique ability to receive, integrate, store, and send information. Signals received from the internal and external environment are processed by neurons, resulting in the generation of a response that can be communicated to other neurons or tissues. In this way, the organism can successfully adapt to rapidly changing events, ensuring its survival.

All organisms possess stimulus-response systems that permit them to sense environmental fluctuations. In bacteria, for example, intracellular regulatory molecules couple the stimulus to the proper response. Multicellular organisms must communicate information to other cells, some of which may be local, but others are positioned some distance away. Communication may be accomplished by the release of chemical messengers that bind to specific complementary proteins called receptors, located on the surface of other cells. For local communication, diffusion can deliver the messenger to the receptive surface. Messengers such as hormones, secreted by endocrine cells in response to

changes in the internal milieu, may have to travel long distances through the bloodstream to reach their targets. This voyage takes time—seconds, hours, or even days—and hormones are, in general, considered slow-acting agents, although evidence is emerging for rapid effects of hormones, such as for the steroid hormones estrogen and corticosterone. Because hormones are diluted in the bloodstream, they must be very potent and act at low concentrations to be effective. These properties are sufficient for endocrine functions necessary to keep the organism in a homeostatic state, but more immediate challenges require a rapid coupling of stimulus and response and a faster rate of communication among relevant cells. Rapid communication is achieved exquisitely by the neuron, whose form and function are designed to meet such demands.

The rapidity with which neurons can conduct signals (i.e., time is measured in less than 1 ms) is primarily a function of certain basic features common to all neurons: polarization of their form, unique associations with their neighboring neurons, and special properties of their plasma membranes. Information is conveyed within and between neurons in the form of electrical and chemical signals, respectively. Neurons are organized into complex networks, or functional circuits, which translate these signals into the myriad responses that constitute an organism's behavioral repertoire. Neural circuits develop in a predictable manner, achieving organizational specificity at functional sites

of contact called *synapses*. At the synapses, neurons transmit signals with a great degree of fidelity, allowing some behaviors, particularly those necessary for survival, to be stereotyped. Neurons also possess a remarkable ability to modify the way messages are received, processed, and transmitted and may exert profound changes in behavioral patterns. This special property is called *plasticity* and depends on the molecular and structural properties of the neuron.

In addition to forming specific contacts with other nerve cells, neurons exist in relation to a group of cells collectively known as *glia*. Some glial cells envelop

neurons and their processes and appear to provide them with mechanical and metabolic support. Others are arranged along specific neuronal processes so as to increase the rate of conduction of electrical signals. Glia are considered later in this chapter. We first focus on the neuron, which is the fundamental structural and functional unit of the nervous system.

1.1. Synapses Are the Sites of Directed Communication Between Neurons

The polarization of neuronal shape permits its functioning within a simple or complex circuit. Like other

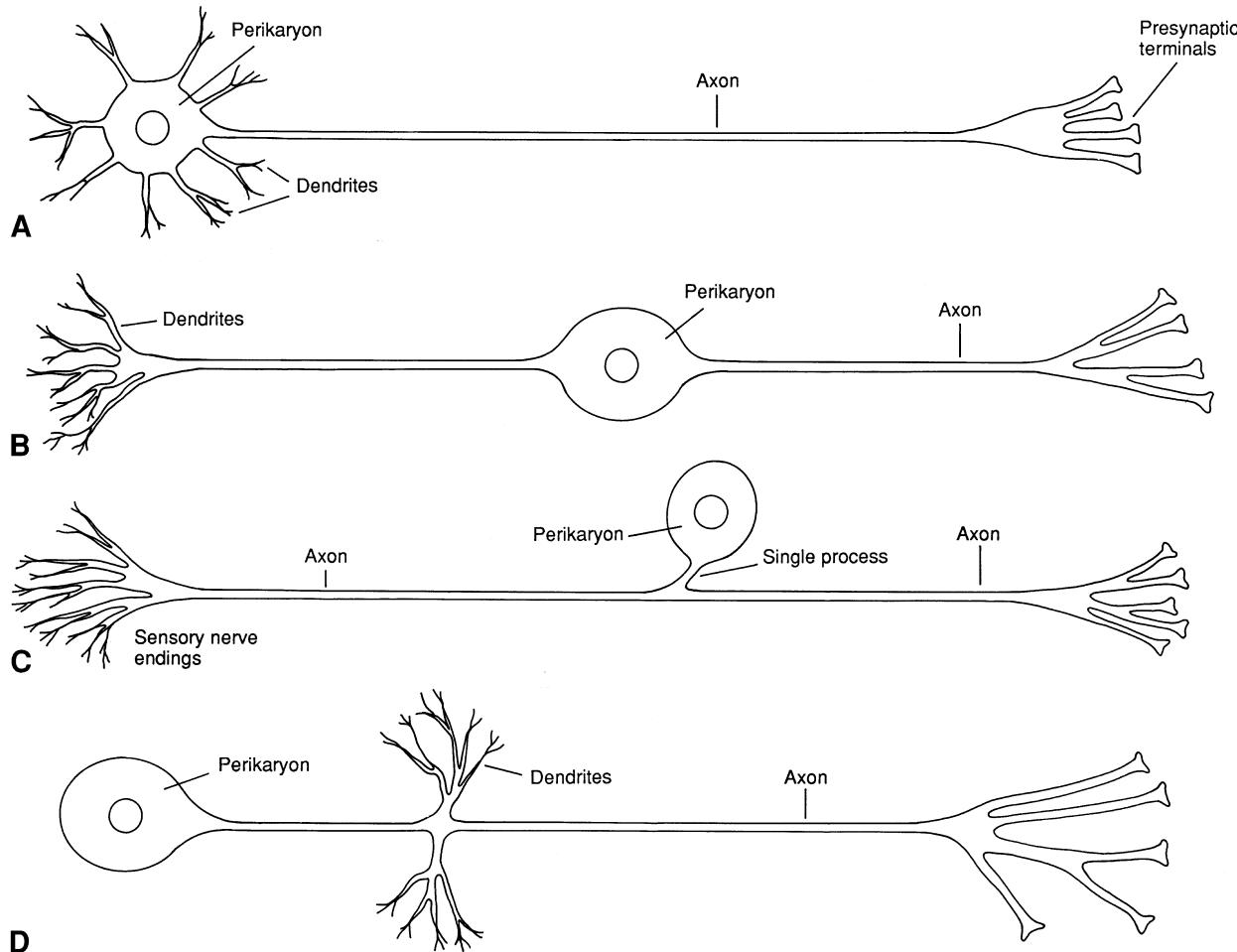


Fig. 1. Polarization of the neuronal form. Nerve cells conduct impulses in a directed manner, although the position of axons and dendrites relative to the cell body and to each other may vary. **(A)** The multipolar neuron is the most prevalent type and shows extensive branching of the dendrites that emanate from the cell body. The axons emerge from the opposite end. **(B)** Bipolar neurons have sensory functions and transmit information received by dendrites along two processes that emerge from the cell body. **(C)** Pseudounipolar neurons are found in the dorsal root ganglion. These neurons are bipolar during early development. Later, the two processes fuse to form a stalk, which subsequently bifurcates into two axons. The action potentials are conducted from sensory nerve endings in skin and muscle to the spinal cord. The action potential usually bypasses the cell body, but in conditions such as a prolapsed vertebral disk, in which the dorsal nerve roots are put under pressure, the cell bodies may also generate impulses. **(D)** Unipolar neurons are found in invertebrates. Axons arise from the dendrites, which emerge from the cell body.

cells, neurons possess a cell body, or perikaryon, which is the metabolic hub of the cell. However, cellular processes extend from this center and give the neuron its unique form and ability to receive and rapidly send signals often over long distances (Fig. 1).

Signals are communicated between neurons at synapses. One neuron forms the presynaptic element, and the subsequent neuron forms the postsynaptic element. Chemical signals, in the form of neurotransmitters or neuropeptides, are concentrated at the presynaptic site. The postsynaptic site contains a high concentration of receptor molecules that are specific for each messenger. Presynaptic and postsynaptic elements are separated by a space of only 20 to 40 μm , ensuring precise and directed transmission of signals.

1.2. Neuronal Polarity Is a Function of Axons and Dendrites

The two main types of cellular processes are called dendrites and axons (Fig. 1). *Dendrites* are usually postsynaptic and form an enormous receptive surface, which branches extensively. In some neurons, such as cerebellar Purkinje cells, the dendritic branches form a characteristic elaborate arborization; others are less distinctive. In addition to their growth during normal development, some of these processes remain plastic and can change in length dramatically in the adult. For example, the extent of arborization of the dendritic tree of male rat motor neurons that innervate penile muscles and mediate copulatory behavior appears to be under steroid hormone (i.e., androgen) regulation. Stress and stress hormones appear to decrease dendritic length in the hippocampus, a brain area involved in memory and learning and responses to stress. In some dendrites, the membranous surfaces are further elaborated to form protrusions called dendritic spines. Chemical signals received by dendrites and their spines are integrated and transduced into an electrical signal, known as the *synaptic potential*.

The signal triggers the action potential, which is propagated along the neuron's plasma membrane down an elongated process called the *axon*. Although axons are not as expansive as dendrites, they branch and may innervate more than one effector. The terminal end of an axon is modified to form a bulbous structure, the *presynaptic terminal* (Fig. 1). The incoming action potentials cause the release of neurotransmitter molecules that bind to complementary postsynaptic receptors. This binding initiates the

other type of electrical signal, the synaptic potential at the postsynaptic site. The unidirectional or polarized flow of information consists of action potentials at the axonal level eliciting synaptic potentials in the postsynaptic cell, which triggers an action potential in that cell and so on. Axons may be as long as 2 m, permitting long-distance and rapid communication within the circuit.

1.3. Diversity in Form Is a Distinctive Property of Neurons

Although neuronal form follows the basic plan described above, nerve cells show a tremendous diversity in size, shape, and function, which allows them to discriminate the multitude of different types of incoming signals. The detection of commands for muscle contraction is under the control of motor neurons. Information in the form of light, mechanical force, or chemical substances is distinguished by sensory neurons highly specialized for each particular type of sensation. It is perhaps in this group of neurons that structural and functional diversity is most apparent. The rigorous demands of sensory discrimination have imposed on these neurons the requirement to develop specific, highly sensitive detection systems that are able to perceive various degrees of stimulus intensities. A classic example is the olfactory cell of the male gypsy moth, which can detect a molecule of the female's sex attractant, or pheromone, released a mile away. Equally impressive is the ability of the mammalian nasal epithelium to detect and discriminate more than 10,000 different odiferous substances. In terms of the ability to recognize diverse molecules, olfactory neurons are second only to the cells of the immune system. The olfactory neuron, however, is markedly different in form and receptor locale from, for example, the retinal photoreceptor cell. Receptive surfaces consisting of specialized cilia on olfactory neurons contain the receptors and are the primary sites of sensory transduction. In retinal photoreceptor neurons, visual transduction occurs within special cylindrical cellular domains containing stacks of about 1000 membranous disks in which the photon receptors called rhodopsin are embedded.

Sensory and other types of information enter the nervous system by means of a particular neuron, but those signals are rarely sent to the effector neuron surfaces directly. Rather, the initial signal is sent to a third class of nerve cell, the *interneurons*. Interneurons integrate various inputs from other neurons before the information, often in a modified form, reaches its final destination. In this way, various

types of inputs (e.g., sensory, hormonal) can be integrated and relayed to other parts of the central nervous system or to motor or endocrine targets. Interneurons contribute to the formation of neural circuits and are to a great extent responsible for the relatively large size and extraordinary complexity of the mammalian nervous system.

1.4. Neuronal Polarity Allows the Directed Flow of Electrical and Chemical Signals

A single neuron may receive a multitude of different inputs from a variety of sources. A motor neuron, for example, receives thousands of presynaptic terminals from many different neurons on its dendritic surface and on its somal and, to some extent axonal, membranes, which may also bear postsynaptic receptors. This input has to be organized into a cohesive message that can be transmitted to its postsynaptic neighbor.

The neuronal plasma membrane plays a key role in integrating and relaying the information in a directed manner and with exceptional speed. Information is conducted within neurons in the form of electrical signals, which are actually changes in the distribution of electrical charges across the neuronal membrane. Charge distribution is highly regulated in neurons by specific and selective proteins called ion channels embedded in the plasma membrane. These transmembrane proteins control the flow of ions and, consequently, the distribution of positive and negative charges across the membrane. In resting neurons, the membrane potential is about -60 to -70 mV (i.e., an excess of positive charges outside and negative charges inside). When this potential becomes less negative, or depolarized, electrical excitation occurs in the membrane. In axons, this excitation is known as the *action potential*. The action potential is generated when the membrane potential of the axonal membrane is decreased beyond a threshold value. An important area of the cell body is the *axon hillock*, the site of summation of excitatory and inhibitory input. Structurally, it is devoid of organelles, such as rough endoplasmic reticulum (*see* later) and, at the light microscopic level, appears as a lightly stained area. After summation, the membrane potential reaches its threshold, thereby generating the action potential.

2. MECHANISMS OF NEURONAL FUNCTION

Compared with other cells, neurons are unsurpassed in their complexity of form and ability to communicate with lightning speed over long distances.

Nevertheless, as eukaryotic cells, they adhere to basic laws that govern cellular function. In many ways, neurons are not so different from other cells, and qualities once ascribed only to neurons may be found elsewhere. Egg membranes, for example, can depolarize during fertilization and release granules in response to Ca^+ entry through specific channels, although the process takes much longer than comparable signaling mechanisms in neurons. Sites of ribosomal RNA synthesis, called nucleoli, are found in all eukaryotic cells but are especially prominent in neurons, because there is a constant need for ribosomes for new protein synthesis. It appears that basic cellular mechanisms are present in nerve cells but that some of these are amplified to meet the rigorous demands of neuronal form and function.

2.1. The Nucleus Is the Command Center of the Neuron

The molecular and cellular diversity within and among neurons reflects a highly controlled differential expression of genes. Gene regulation also determines nerve cell connectivity, which dictates patterns of stereotyped behaviors. It is also becoming evident that more complex behaviors, such as memory and learning, may require the synthesis of new proteins, which ultimately depends on the expression of particular genes. As eukaryotic cells, neurons sequester their genome in the nucleus, the largest and most conspicuous feature of the perikaryon (Fig. 2). The nucleus contains chromosomal DNA and the machinery for synthesizing and processing RNA, which is subsequently transported to the cytoplasm, where information encoded in the DNA is expressed as specific proteins. The nucleus is separated from the rest of the cytoplasm by a porous double membrane, the *nuclear envelope*, consisting of an *outer nuclear membrane* and an *inner nuclear membrane*; the inner and outer membranes are in contact with each other at regions called *pore membranes*, which are described below. Beneath and in intimate contact with the inner nuclear membrane is the *nuclear lamina*, consisting of intermediate filaments, and which controls such functions as the maintenance of nuclear shape, disassembly and assembly of the nucleus prior to and following mitosis, organization of the chromatin, spacing of nuclear pores, and transcriptional regulation. At least 13 genetic disorders involving genes encoding some of the laminins have been described and include premature aging syndromes, myopathies and neuropathies, and lipodystrophies. The nuclear envelope protects the DNA molecules from mechanical

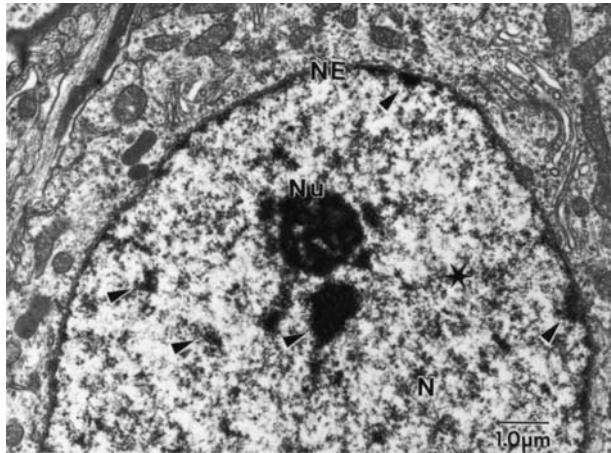


Fig. 2. Electron micrograph of a nucleus of a hypothalamic neuron. The nucleus (N) is separated from the cytoplasm by a nuclear envelope (NE). A conspicuous nucleolus (Nu) signifies a great demand for ribosomal RNA by the neuron. Proteins called histones associate with DNA to form chromatin, which may appear extended (i.e., euchromatin) or condensed (i.e., heterochromatin), depending on the translational activity of specific regions of the genome. Most of the nucleus contains fine fibers of euchromatin (asterisk). Heterochromatin (arrowheads) is seen as clumps within the nucleus, on the inner surface of the nuclear envelope, or associated with the nucleolus.

perturbations caused by cytoplasmic filaments. Moreover, it separates the process of RNA synthesis (i.e., transcription) from that of protein synthesis (i.e., translation). This segregation of function has an important advantage over the situation in prokaryotes, in which transcription and translation occur simultaneously. In these organisms, protein synthesis begins before the completion of transcription, limiting the opportunity for modifying the RNA. Translation in eukaryotes does not begin until the RNA is transported into the cytoplasm. In the nucleus, the RNA may be modified in such a way that specific portions of the RNA molecule are removed (i.e., RNA splicing) or altered. These complex changes have important implications for cell function. Mechanisms that control variability at the level of transcribed RNA allow a single gene to code for several different proteins, resulting in the rich diversity seen, especially in neurons, in the form of neuropeptides, receptors, ion channels, and cytoskeletal proteins.

2.1.1. CHROMATIN STRUCTURE IN THE REGULATION OF GENE ACTIVITY

The degree of complexity of gene expression is further multiplied at another, even more fundamental level of gene regulation: the DNA. Genes are turned on by

complexes of proteins called *transcription factors*. Each of these proteins possesses special DNA-binding domains and requires direct contact with the DNA to function. However, the long stretch of DNA, which in humans measures about 3 cm long, is folded thousands of times to fit into a nucleus only a few micrometers in diameter. Such compaction creates a potential problem for factors that must gain free access to corresponding binding sites and other regulatory regions on the DNA strand. Another group of proteins, called *histones*, packs the DNA so that it is folded, coiled, and compressed many times over to form fibers called *chromatin*, visible as fine threads in the interphase nucleus (Fig. 2) and, in an even more contracted form, as chromosomes in the dividing cell.

In the mature neuron, the nucleus remains in interphase. DNA is segregated into morphologically distinct areas, reflecting the degree of chromatin condensation, which is a function of nuclear activity (Fig. 2). Ribosomal DNA genes and their products are separately packed into a structurally defined compartment called the *nucleolus* that is specialized for ribosomal RNA synthesis. Highly coiled regions for the genome appear as dense, irregularly shaped clumps known as *heterochromatin*. These areas of condensed chromatin are situated within the nucleus along the inner nuclear membrane, in association with the nucleolus or dispersed within the nucleus proper. Other regions of the genome readily available for transcription into messenger RNA appear as fine filaments and are known as *euchromatin*.

2.1.1.1. Chromatin Remodeling in Behavior and in Neural Diseases. The manner in which DNA encodes proteins is discussed elsewhere. These proteins ultimately affect our behavior. However, novel studies reveal the exciting possibility that our behavior may modify the genome and that some of these changes may become permanent. These changes are known as *environmental programming* and are part of the phenomenon of *epigenesis*, whereby stable and heritable alterations in gene expression are not directly due to changes in DNA sequences. An example is that of maternal behavior in rats. In rodents, maternal behavior, such as tactile stimulation by the mother toward the pup, can result in increased amounts of specific second messengers and gene transcription factors. Binding of certain transcription factors to the DNA may result in the recruitment of a class of enzymes, called *histone acetyltransferases*. These enzymes increase histone acetylation, which permits

access of another enzyme, *demethylase*, allowing demethylation of a promoter to facilitate the recruitment of DNA-binding proteins. These proteins, in turn, may facilitate the expression of beneficial genes. Conversely, in the absence of tactile stimulation and increased transcription factor, the promoter will remain methylated. An example of one of these promoters is that for the glucocorticoid receptor, which appears to be involved in modulating stress. Other behavioral studies suggest that handling of newborn rats decreases the extent of the hormonal response to stress in adulthood; that is, the rats are less stressed as adults. Importantly, the experience of tactile stimulation by the mother may be converted into a phenotypic variation of the offspring that may be transmitted across generations. This phenomenon may translate into human behavior, where the kind of parental care received by children can impact physiologic responses to stress (resulting, for example, in cognitive and behavioral problems) that persist into adulthood. Epigenetic modifications can also result in various brain dysfunctions, including Rubinstein-Taybi syndrome and the Coffin-Lowry syndrome, disorders characterized by mental retardation, and the α -thalassemia/mental retardation syndrome.

2.1.2. NUCLEOLUS AS THE SITE OF RIBOSOMAL RNA SYNTHESIS

The nucleolus is a prominent spherical region of the nucleus (Fig. 2) containing that portion of the genome dedicated to the transcription of ribosomal DNA and the mechanisms for the assembly of ribosomal subunits, the precursors of mature ribosomes. Large precursor ribosomal RNA molecules are processed in the nucleus, resulting in the degradation of almost half of the nucleotide sequences. The remaining ribonucleoprotein molecules form two subunits that are independently transported into the cytoplasm, where the mature ribosomes are assembled. The nucleolus is evident only in the interphase nucleus; in other cells that undergo mitosis, it decondenses, ribosomal RNA synthesis stops, and ribosomal DNA genes associate with specific regions of the chromosomes called *nuclear organizing regions*.

2.1.3. NUCLEAR PORE COMPLEX CONTROLS TRAFFIC BETWEEN THE NUCLEUS AND CYTOPLASM

The double membrane comprising the nuclear envelope presents a formidable barrier between the nucleus and cytoplasm. Macromolecular traffic into and out of the nucleus is achieved by perforations, or *pores*, in the nuclear envelope. At various points

along the envelope, the inner and outer membranes are in continuity around the edges of each pore at a region called the pore membrane (Fig. 3). The nuclear pore is not a simple opening. The *nuclear pore complex*, itself, resembles a megaphone, with the large opening facing the cytoplasm and the small opening on the nuclear side. The basic structure consists of *cytoplasmic fibrils*, a central core, and a *nuclear cage* or basket. Depending upon the organism, from 50 to 100 different proteins, called *nucleoporins*, or *Nups*, make up the entire structure. Irregardless of the organism, however, there are only about 30 distinct nucleoporins. Nucleoporins are involved in binding large transported molecules (displaying diameters of up to 39 nm), called cargo, trapping the molecules, and terminating the transport reaction. Other proteins, called karyopherins (Kaps), are shuttling transport, or carrier, proteins. Karyopherins, including *importins* and *exportins*, recognize *nuclear localization* or *export signals* on the cargo, which allow entry into or export from the nucleus, respectively. The transport is energy

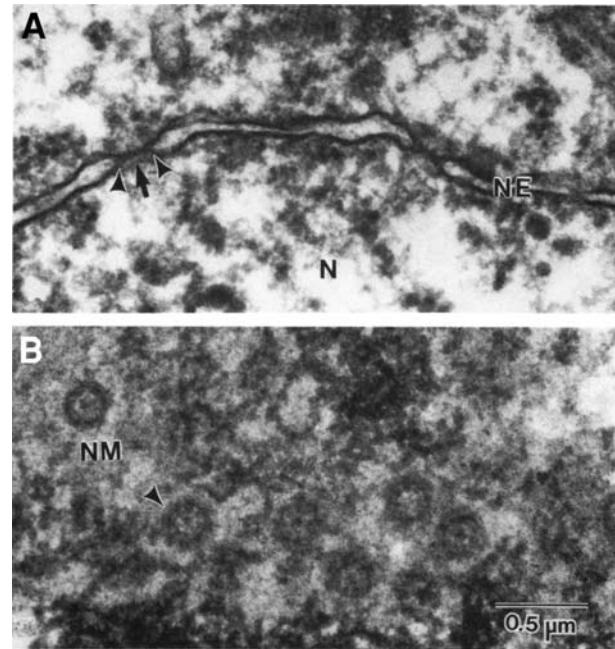


Fig. 3. Electron micrographs of nuclear pores. **(A)** The perpendicular section of the nuclear envelope (NE) shows the continuity of the inner and outer nuclear membranes (arrowheads) around a nuclear pore (arrow) of the nucleus (N). **(B)** A surface view shows the arrangement of nuclear pores in the nuclear membrane (NM). One of the nuclear pores (arrowhead) shows the octagonal configuration of proteins comprising the nuclear pore complex. **0.5 μ m**

dependent for both import and export. The enzyme GTPase or *Ran* provides energy and cycles between a guanosine triphosphate (GTP)- and guanosine diphosphate (GDP)-bound state, with Ran GTP being primarily nuclear and Ran GDP primarily cytoplasmic in location. Small molecules (less than 40 kDa), ions, and metabolites can cross the nuclear pore complex by passive diffusion. Some proteins associated with the inner nuclear membrane itself are thought to be imported both passively and actively. Some particles, such as the assembled mature ribosomes, are too large to gain entrance through these passageways, ensuring that protein synthesis is restricted to the cytoplasm.

2.1.4. DYNAMIC NUCLEAR MORPHOLOGY REFLECTS ALTERATIONS IN THE GENOME

Although nuclear events occur on a molecular scale, they may be detected by gross adjustments in nuclear morphology. The overall size and shape of nuclei and nucleoli can change with the metabolic and physiologic demands of the neuron. Depending on transcriptional activity, various segments of the chromatin can condense or decondense, resulting in a relative change in the disposition of heterochromatin and euchromatin and altering the general appearance of the nucleus. For example, in the hypothalamus, a brain area controlling female reproductive behavior, the gonadal steroid hormone estrogen exerts a profound influence on nuclear morphology, altering the size, shape, and position of heterochromatic regions. Nucleolar size is also subject to the physiologic conditions of the cell. Estrogen treatment has a pronounced effect on precursor ribosomal RNA levels, which are accompanied by a significant increase in nucleolar area in the hypothalamus of ovariectomized animals. Nucleolar hypertrophy is followed by a massive increase in rough endoplasmic reticulum in these neurons.

2.2. Neurons Are Actively Engaged in Protein Synthesis

Information contained within the genome is expressed as biologically active peptides in the cell body or perikaryon of the neuron. Some peptides are neuron specific, such as some of the neurosecretory peptides, cytoskeletal proteins, ion channels, and receptors. Others are common to all cells and are involved in increasing the efficiency of transcriptional and translational events related to the production, transport, and release of these proteins.

2.2.1. PROTEINS SYNTHESIZED BY NEURONS FOR EXPORT

Appreciation of the tremendous protein synthetic activity of the nerve cell and its functional implications is relatively recent. Neuronal form and membrane properties were the main focus of earlier neurobiologists. However, the compelling discovery of glandular cells in the spinal cord of fish by Carl Speidel in 1919 and of neurosecretory cells in the hypothalamus by Ernst Scharrer in 1928 directed attention to the great degree of biosynthetic activity in the perikaryon. Ernst Scharrer noticed that the secretory activity of diencephalic neurons was comparable with that seen in endocrine cells. This observation stimulated further interest in finding structural counterparts of the secretory process in neurons. The mechanisms involved in peptide biosynthesis and posttranslational processing are now well-known. Related molecular and biochemical events are detailed in other chapters of this volume. In this chapter, we describe the structural correlates of these functions as they occur in various parts of the nerve cell.

2.2.1.1. *Synthesis of Exportable Neuropeptides on the Rough Endoplasmic Reticulum.*

Neurons must transmit chemical information and electrical signals over very long distances. Proteins are synthesized, packaged, processed, stored, and released in different domains of the neuron. The synthesis of exportable proteins begins in the perikaryon. Preribosomal subunits produced in the nucleolus enter the cytoplasm, where they are assembled and activated to form functional ribosomes. In some cases, they attach to membranous cisternae comprising the rough endoplasmic reticulum (Fig. 4). The outer nuclear membrane ramifies within the cytoplasm as it encircles the nucleus and, studded with ribosomes, also becomes part of the protein synthetic apparatus.

Messenger RNAs associated with ribosomes are translated into precursor proteins. The precursors are larger than the biologically active peptides and must be enzymatically cleaved and modified to attain their final form. Instructions about whether a given protein is destined for export are also encoded in the DNA of its precursor. A portion of the complementary messenger RNA is translated into a *signal peptide*, which directs ribosomes to the cisternae of the rough endoplasmic reticulum. Peptides lacking a signal sequence cannot gain entry to the cisternae. The disposition and extent of rough endoplasmic

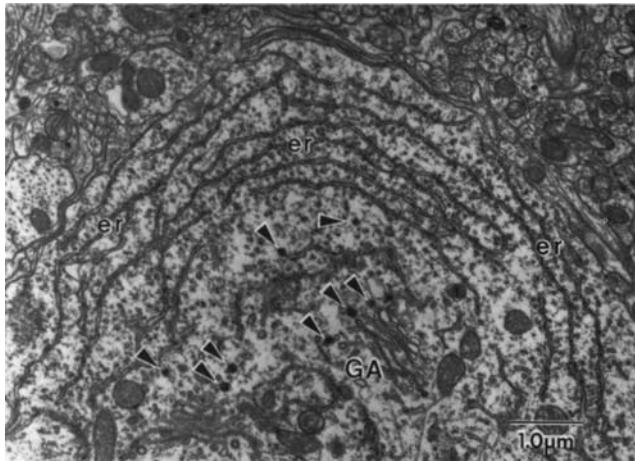


Fig. 4. Electron micrograph of a hypothalamic neuron actively engaged in the synthesis and packaging of exportable proteins. Many cisternae of rough endoplasmic reticulum (er) are arranged in parallel stacks. The Golgi apparatus (GA) and associated dense-cored vesicles (*arrowheads*) are also visible. (From Cohen RS, Pfaff DW. Ultrastructure of neurons in the ventromedial nucleus of the hypothalamus with or without estrogen treatment. *Cell Tissue Res* 1981;217:463.)

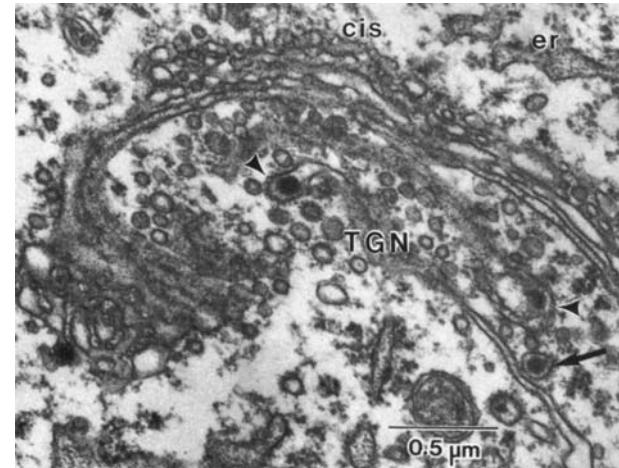


Fig. 5. Electron micrograph of a Golgi apparatus of a hypothalamic neuron. Small vesicles surround the Golgi apparatus on all sides. The cis face is located near the rough endoplasmic reticulum (er); the opposite side is designated as the trans Golgi network (TGN). Secretory material is detected within the peripheral portions of the cisternae (*arrowheads*) of the TGN. A dense-cored vesicle (*arrow*) containing material of similar density is seen at the periphery of the TGN.

reticulum vary and appear to depend on the prevailing demands for secretory protein synthesis in a functional group of neurons. In nerve cells that are quiescent in regard to the production of exportable proteins, cisternae of the rough endoplasmic reticulum appear as discrete sacs and seem to occupy only a small fraction of the cellular space. Neurons actively engaged in secretory protein synthesis contain large stacks of elongated cisternae that fill a considerable portion of the perikaryon.

2.2.1.2. Packaging and Modification of Exportable Neuropeptides in the Golgi Apparatus. The Golgi apparatus is composed of a series of flattened, smooth-surfaced, membranous sacs and a variety of associated small vesicles, which encompass the Golgi apparatus on all sides (Fig. 5). The Golgi cisternae are arranged in a polarized fashion, reflecting their morphology and function. The forming or cis face approaches the rough endoplasmic reticulum; the opposite side is designated as the maturing or trans face or trans-Golgi network. Vesicles bud off from the smooth transitional portion of the rough endoplasmic reticulum and transport their contents to Golgi sacs on the cis face. Here, they deliver the newly synthesized precursor protein by fusion of the vesicle membrane with the Golgi membrane.

The secretory material is later detected within the cisternae occupying the trans-Golgi network. Often, at the periphery of the sacs, the membranes appear to constrict around a dense granule, which is the concentrated precursor protein (Fig. 5). The granule-containing membranous compartment is pinched off, forming a dense-cored vesicle. Although the details of protein processing within the Golgi apparatus have not yet been elucidated, its overall function is known: the preparation and packaging of the secretory protein, still in its precursor form, for transport to the axon terminal for storage or release.

2.2.1.3. Formation of the Active Neuropeptide Within the Neurosecretory Vesicle. The actual enzymatic processing of the precursor and generation of individual biologically active peptides appear to occur distal to the Golgi apparatus as the secretory vesicle makes its way down the axon. Conditions, such as pH, necessary for the maximal activity of the processing enzymes, are optimal. The relatively simple structure of the secretory vesicle—a dense core surrounded by a single membrane—betrays its dynamic role in generating active neuropeptides. Various enzymatic activities have been located in neurosecretory vesicles, and some have been purified and characterized.

The vesicle membrane contains proteins that regulate the internal vesicular environment. On the appropriate signal, active neuropeptides are released by exocytosis, which is fusion of the vesicle membrane with the plasma membrane at the presynaptic site.

2.2.2. PROTEINS SYNTHESIZED FOR USE WITHIN THE NEURON

The synthesis of exportable proteins represents only a portion of the total protein synthetic effort by the neuron. The elaboration of the cytoskeletal framework and the synthesis of other proteins including ion channels, receptors, second messenger systems, and other proteins destined for maintenance and renewal of the cytoplasm and its organelles also depend on translation of messenger RNAs. These proteins are synthesized on free ribosomes that are plentiful in all nerve cells. Axons possess a highly organized transport system to convey proteins to presynaptic terminals or back to the cell body. Dendrites, their postsynaptic specializations, and dendritic spines also require proteins for growth, maintenance, and function. Although there is an indication of molecular transport to these sites, the identities of the molecules and the nature of the transport mechanism has just begun to be explored. Moreover, evidence for local protein synthesis suggests that not all dendritic and postsynaptic proteins arrive from the cell body. The presence of polyribosomes beneath postsynaptic membrane specializations and at the base of dendritic spines provides the machinery for local protein synthesis. The existence of local protein synthetic mechanisms is further supported by the presence of some messenger RNAs in distant dendritic arbors, suggesting that growth-dependent and activity-dependent synaptic alterations, including changes in morphology, may be regulated partially by the local synthesis of key synaptic proteins.

2.3. Smooth Membrane Compartments in Neurons Serve as Reservoirs for Calcium

In addition to the intramembranous compartments that directly participate in the synthesis, packaging, and transport of secretory peptides, other cisternal and vesicular structures are evident within the various domains of the neuron. The membranous sacs are visible as smooth-surfaced compartments in a variety of configurations. Some appear as relatively short sacs, but others are longer and anastomose within neuronal processes. In the cell body, smooth membrane profiles are arranged in stacks or emanate from

cisternae of the rough endoplasmic reticulum. One of the most morphologically complex variations of these structures resides in the dendritic spine, where parallel, smooth-surfaced cisternae alternate with electron-dense bands of unknown composition.

All of the diverse structures are thought to function in the *release and sequestration of calcium* within the neuron. Calcium is central to most aspects of neuronal function, including membrane permeability; mediation of the effects of neurotransmitters, hormones, and growth factors; cytoskeletal function; and vesicle release. In neurons, calcium mobilization is achieved, at least in part, by the binding of inositol 1,4,5-triphosphate (IP) to intracellular receptors located on the aforementioned membranous cisternae. IP is a second messenger generated on receptor-stimulated hydrolysis of phosphatidylinositol 4,5-biphosphate by phospholipase C, an enzyme activated by signal transduction mechanisms.

2.4. Mitochondria

2.4.1. MITOCHONDRIAL STRUCTURE IN NEURONS

Mitochondria from various brain regions and neuronal compartments (perikarya, dendrites, axons, and synapses) have essentially uniform membrane architecture. Mitochondria in neurons contain interconnected tubular and lamellar cristae, with the tubular-shaped cristae arranged more peripherally and the lamellar ones located more centrally. The functional significance of these features is unknown, although changes in cristae shape may contribute to regulation of ATP production. The outer mitochondrial membranes are also in close association with membranes of the endoplasmic reticulum, at sites of high calcium generation. Some neurons display a higher accumulation of mitochondria in the soma, the axon hillock, the nodes of Ranvier, and the nerve terminal; during neuronal development, mitochondria are also located in growth cones.

2.4.2. MITOCHONDRIAL FUNCTION IN NEURONS

Mitochondria are present in all cells and provide common functions irrespective of the particular cell type. These functions include the generation of ATP, as well as reactive oxygen species, intermediary metabolism, intracellular calcium signaling, and the regulation of apoptosis. However, the unique compartmentalization of neurons into different structural and functional domains and their high-energy requirements necessitate an expanded role

for mitochondria in these cell types, whereby mitochondria affect nerve transmission and vice versa. During development, mitochondria appear to function as a determinant of neuronal polarity, in the control of neurite outgrowth, and the differentiation of neurons from precursor cells. They also play a role in adult plasticity by influencing neurotransmitter release from presynaptic terminals, possibly via their role in calcium signaling. On the postsynaptic side, mitochondria appear to affect the sensitivity of neurons for glutamate. Notably, environmental factors, which may also influence plasticity, appear to affect mitochondria. Rats kept in an enriched environment, resulting in enhanced performance of a spatial memory task, display an increase activity of some mitochondrial proteins. That synaptic activity can alter mitochondrial biochemistry and function is evidenced by the upregulation of mitochondrial genes by high-frequency stimulation of a tissue slice from the hippocampus.

In terms of ATP, many neuronal functions require energy, including those functions associated with the cytoskeletal proteins, as well as those involving phosphorylation. Calcium influx and its sequestration and release are also essential to neuronal function, and mitochondria are also key players here. The production of reactive oxygen species by this organelle may be involved in cell signaling, as well as membrane lipid peroxidation, which, in turn, alters membrane protein function.

Mitochondria play a role in *apoptosis*, a type of programmed cell death. Part of this process is mediated by Bcl-2 family members, which interact with mitochondrial membranes to either increase or decrease their permeability, resulting in apoptosis or, alternatively, stabilize the membrane to check this process. During the process of apoptosis, mitochondrial membranes exhibit increased permeability and release *cytochrome c*. Cytochrome c itself can activate *caspase-3*, which, in turn, may cleave some protein substrates resulting in cell death. On the other hand, when activated at sublethal levels, some caspases in synapses and dendrites may cleave specific glutamate receptor subunits, thereby modulating synaptic plasticity.

GTP binding protein-coupled receptors for neurotransmitters and neuropeptides, glutamate, and neurotrophic factors can also affect mitochondria via second-messenger pathways. These pathways target gene transcription factors, with the possibility of encoding proteins relevant to synaptic and neuronal plasticity.

2.4.3. MITOCHONDRIA AND NEURODEGENERATIVE AND PSYCHIATRIC DISORDERS

Mutations in mitochondrial DNA may result in the maternal transmission of neurologic disorders. These appear to include some cases of schizophrenia, some neuropathies, and retinitis pigmentosa. Mitochondrial dysfunction may also be involved in Alzheimer's and Parkinson's diseases, as well as stroke. In these cases, age-related changes in neuronal metabolism as a consequence of mitochondrial dysfunction may indicate prior age-related changes in calcium balance and interfere with synaptic plasticity. Moreover, accumulation of cytotoxic forms of certain proteins implicated in Alzheimer's disease may result in mitochondrial dysfunction and/or displacement. For example, defects in the human presenilin 1 gene, which is implicated in an aggressive form of early-onset familial Alzheimer's disease, appear to compromise kinesin-based axonal transport in neurons. Kinesin is a molecular motor important in anterograde axonal transport. Neurons with the mutant form of presenilin 1 display reduced mitochondrial density in neuritic processes. In regard to other psychiatric disorders, patients with bipolar disorder appear to shift their metabolism toward glycolytic-based energy production, as opposed to one that involves oxidative phosphorylation. Moreover, some patients with schizophrenia display fewer mitochondria in specific brain regions, and neuroleptic drugs appear to reverse this phenomenon.

3. CYTOSKELETON DETERMINATION OF NEURONAL FORM

A singular feature of neurons is their overall extraordinary length, enabling them to transmit signals over great distances. This property is reflected in the polarity of neuronal form and function, which is governed by regional specialization of the plasma membrane and by differences in the cytoskeletal composition of dendritic and axonal processes emerging from the cell body. Although the neuronal cytoskeleton provides a structural framework on which various organelles and cellular events are organized, it is by no means a static configuration. Throughout the neuron, molecular alterations in cytoskeletal proteins reverberate as microscopically visible changes in movement of the cytoskeleton, its associated organelles, and the shape and extent of some of the processes. Although the cytoskeleton permits the general pattern of individual neurons to remain constant and identifiable, alterations in cytoskeletal dynamics

enable the neuron to respond to environmental-dependent or activity-dependent fluctuations. This apparent contradiction is resolved by the inherent nature of cytoskeletal elements that exist in different structural and functional states of assembly and disassembly. Moreover, these structures may be stabilized and destabilized, providing yet another dimension to the number of possible conformations of cytoskeletal form.

The interactions of various cytoskeletal elements with their associated proteins or with each other contribute to the unique structural and functional identity of axons and dendrites and their associated dendritic spines. The cytoskeleton also interacts with the neuronal plasma membrane at specific sites, including the initial segment of the axon, special loci along the axon called nodes of Ranvier, and presynaptic and postsynaptic membranes, forming complex submembrane filamentous arrays. Such membranous-cytoskeletal associations may restrict the movement of important membrane proteins, such as receptors, at that site or communicate events occurring at the membrane to underlying areas. In this section, we describe components of the neuronal cytoskeleton and how they contribute to the architecture of the neuron and confer specificity to each structural domain.

3.1. The Neuronal Cytoskeleton Provides Internal Support

Axons and dendrites emerge from the perikaryon as delicate strands. Axons may be as much as a million times longer than they are wide. Consequently, these fragile processes require internal support. The rigidity of the cytoskeletal network is apparent after removal of the neuronal membrane with detergents that selectively extract membrane lipids and proteins. In experiments using detergent-treated cultured nerve cells, isolated neuronal processes, and isolated submembranous cytoskeletal patches, the cytoskeleton remains intact, and its shape is virtually identical to its original conformation. The cylindrical form of the axonal cytoskeleton is so cohesive that investigators, using the classic model of the squid giant axon to study axonal transport mechanisms, equate the extrusion of its contents, the axoplasm, to that of toothpaste being squeezed out of its tube. Even isolated submembrane filamentous arrays, such as those found beneath the postsynaptic membrane, appear to retain their curvature after the rigorous processes of homogenization of brain and centrifugation, lysis, and detergent treatment of isolated synaptic

compartments; only after sonication or extremely acidic conditions do these tenacious structures dissociate into their component parts.

3.2. The Components of the Neuronal Cytoskeleton Include Microtubules, Neurofilaments, and Microfilaments and Their Associated Proteins

The dual nature of the neuronal cytoskeleton, reflected in its rigidity and plasticity, is a function of three filament types: microtubules, neurofilaments, and microfilaments or actin filaments. Each cytoskeletal element acts in conjunction with a specific set of associated or binding proteins. Some of these cross-link the filaments to each other, the plasma membrane, and other intracellular organelles and are responsible for the gelatinous and relatively stiff consistency of the cytoskeleton. Other associated and binding proteins affect the rate and extent of filament polymerization, providing a mechanism for localized plastic changes.

Microfilaments consisting of the protein actin are 6 nm in diameter and are prominent in cortical regions, particularly in the highly specialized submembrane filamentous structures, such as the presynaptic and postsynaptic membrane specializations. Microtubules are long, tubular structures that are 25 nm in diameter and form tracks for the transport of various organelles and molecules, although the microtubules are themselves also capable of movement (Fig. 6). The microtubules and actin consist of globular subunits that can assemble and disassemble with relative ease. Neurofilaments that are 10 nm in diameter are a subdivision of the ubiquitous class of intermediate filaments found in all cells (Fig. 6). Mammalian neurofilaments consist of three fibrous subunits that have a very high affinity for each other, and polymers composed of these subunits are very stable. Neurofilament subunits are synthesized and assembled in the cell body and then directed down the axon, where they contribute to its resiliency and its caliber. Neurofilaments are degraded at the entrance to the nerve terminal by Ca^{2+} -activated proteases located at that site.

3.2.1. ACTIN AND TUBULIN POLYMERS

Subunits of actin, a 43-kDa globular protein, and microtubules, a heterodimer of two 50-kDa globular proteins called α -tubulin and β -tubulin, assemble into polymers that bind to identical subunits at each end of a preexisting polymer. The lengths of the polymer are determined by cellular mechanisms that

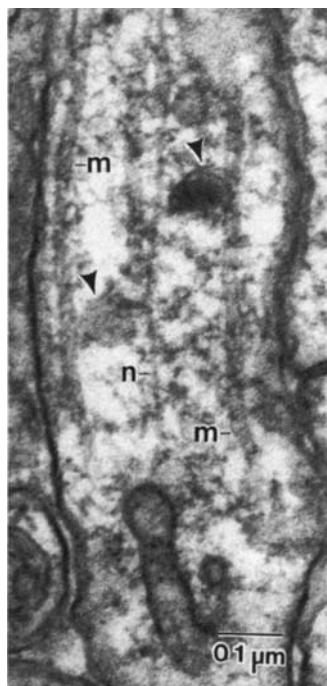


Fig. 6. Electron micrograph of the axonal cytoskeleton. The two prominent cytoskeletal elements in this region are microtubules (m) and neurofilaments (n). The microtubules are 25 nm in diameter and form tracks for the transport of various organelles, such as vesicles (arrowheads). The neurofilaments belong to the ubiquitous class of intermediate filaments and are 10 nm in diameter. Neurofilaments are relatively stable polymers that may contribute to the resiliency and caliber of axons.

control the rates of association and disassociation at the ends of each rod. In some polymers, there is a constant flux of monomers at each end. In more stable polymers, dissociation of the subunits at each end is slow or does not occur at all. Stability can be achieved by blocking the dissociation reaction at either end. Both tubulin and actin monomers are asymmetric so that they can only link up with each other in a specific orientation. Consequently, the resultant polymer is polarized and has plus and minus ends, a feature permitting polymers to grow in a directed manner.

3.2.2. CYTOSKELETAL-ASSOCIATED PROTEINS

Cytoskeletal-associated proteins regulate cytoskeletal structure and function and characterize specific neuronal domains. Purified tubulin monomers can spontaneously assemble into microtubules in the presence of GTP. However, polymerization is greatly enhanced in impure preparations. The impurities are actually a group of accessory proteins that are subdivided into two categories:

microtubule-associated proteins (MAPs) and tau proteins. These proteins induce the assembly and stabilization of microtubules by binding to them. The tau proteins facilitate polymerization by binding to more than one tubulin dimer at the same time. MAPs have two domains, one of which binds to the microtubule and the other to an adjacent MAP molecule, filament, or cell organelle. MAPs provide the neuron with a mechanism for structural plasticity and variability. About 10 kinds of MAPs have been identified, and they appear to be differentially expressed during brain development. Specific MAPs appear to be restricted to different neuronal processes. MAP 2, for example, is expressed in dendrites but not axons (Fig. 7); conversely, MAP 3 is present in axons but not in dendrites. Although microtubules appear in parallel array, actin filaments in neurons are usually visible as a tangled meshwork. The network sometimes appears as a dense submembranous array, as in the *postsynaptic density* (PSD) immediately beneath the postsynaptic membrane. However, the network may be less dense, as in the subsynaptic web immediately beneath the PSD and extending throughout the dendritic spine. Actin filaments are also associated with a group of accessory proteins, the actin-binding proteins, which bundle them or cross-link them to form a gel. Some binding proteins join the filament ends to obstruct further polymerization, and others link actin to the membrane. Actin-binding proteins are regulated by second messengers, such as calcium or cyclic nucleotides.

3.2.3. MOLECULAR MOTORS

Other proteins associated with the cytoskeleton are the molecular motors, which harness energy to propel themselves along filaments. These proteins are enzymes that hydrolyze ATP and GTP and use the liberated energy to move themselves along the polymer. Motion is achieved because each of the steps of nucleotide binding, hydrolysis, and release of ADP or GDP plus phosphate causes a concomitant change in the conformation of the motor protein such that it is directed forward. Myosin motors walk along actin filaments, which are pulled along in the process. This action is important in the motility of growth cones, the pioneering tip of developing nerve cell processes. Motor proteins are also associated with microtubules, where they are involved in organelle transport in neuronal processes.

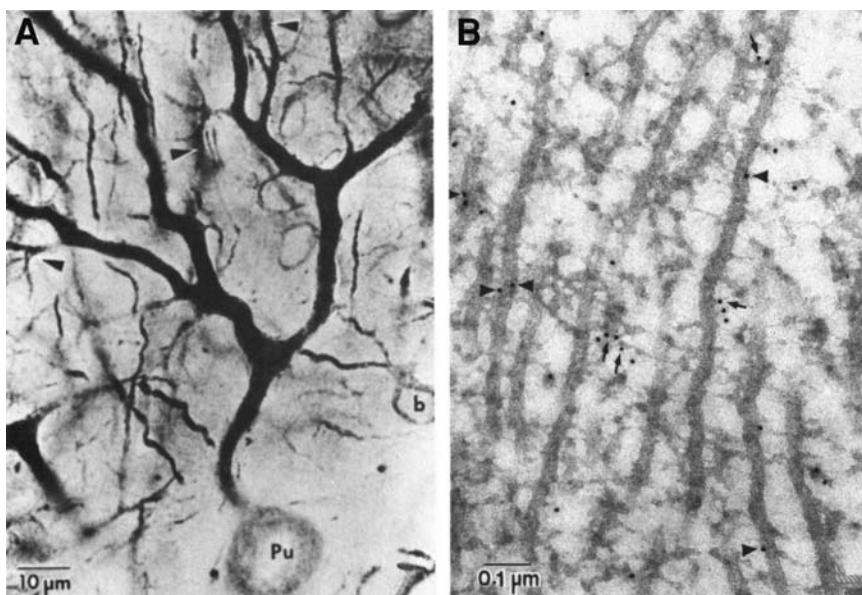


Fig. 7. Dendrites labeled with antibodies to microtubule-associated protein (MAP) 2. The technique of immunocytochemistry uses antibodies to localize specific proteins in neurons. These antibodies are detected by secondary antibodies tagged with an enzyme or gold particle. **(A)** The enzyme, such as peroxidase, catalyzes a reaction that results in an electron-dense precipitate, as seen in the light micrograph, in which antibodies to MAP 2 label the dendritic tree (arrowheads) of a Purkinje cell. **(B)** Alternatively, the secondary antibody can be tagged with gold particles, seen as black dots in the electron micrograph, in which they localize MAP 2 to microtubules (arrowheads) and cross-bridges (arrows) between them. Pu, Purkinje cell; b, basket cell. (Part A is reprinted from Bernhardt R, Matus A. J Comp Neurol 1984; 226:207, with permission of Wiley-Liss, a division of John Wiley & Sons, Inc., New York.)

3.2.4. CYTOSKELETAL-BASED TRANSPORT SYSTEM OF NEURONS

Neuronal processes span great distances to reach their presynaptic and postsynaptic targets, which can be meters away from the cell body in large organisms. Neuropeptides synthesized and packaged in the perikaryon must embark on long journeys to far-removed nerve terminals. Although some synaptic components can be synthesized and recycled locally, others must be imported from the cell body or returned there for degradation by lysosomes, for example. Molecular and organelle traffic to and from these remote areas requires active mechanisms, because diffusion alone would take an inordinate amount of time. Bidirectional traffic in axons delivers proteins and organelles to and from the nerve terminal by anterograde and retrograde transport, respectively. Anterograde transport delivers neuropeptide-containing vesicles and cytoskeletal proteins; retrograde transport returns endocytotic and other vesicles. Axonal transport has two components: a fast component, traveling at rates of 200 to 400 mm/day, and a slow component, which consists of a slow component a (SC_a) and a slow component b (SC_b), moving at rates of 0.2 to 1 mm/day and 2 to

8 mm/day, respectively. The fast component carries membranous vesicles, and the slow compartment carries cytoskeletal proteins.

Fast transport involves movement of vesicles along tracks composed of microtubules. Microtubule motors generate the force required to propel organelles along the path. Two of these motors, kinesin and dynein, are ATPases that are activated on binding to the microtubule. Kinesin directs movement toward the plus end of the microtubule and dynein toward the minus end. In axons, most microtubules have their positive end toward the terminal and can guide movement in either direction. In dendrites, about half of the microtubules are oriented with their plus ends toward the dendritic tip, and the other half have their minus ends toward the tip. Differences in microtubule orientation in axons and dendrites may be one of the mechanisms underlying the selective transport of organelles into dendrites or axons.

4. NEURONAL SYNAPSES

Electrical signals can be conveyed directly from cell to cell at special sites called *gap junctions* (Fig. 8). Ions pass from one cell to another through relatively large

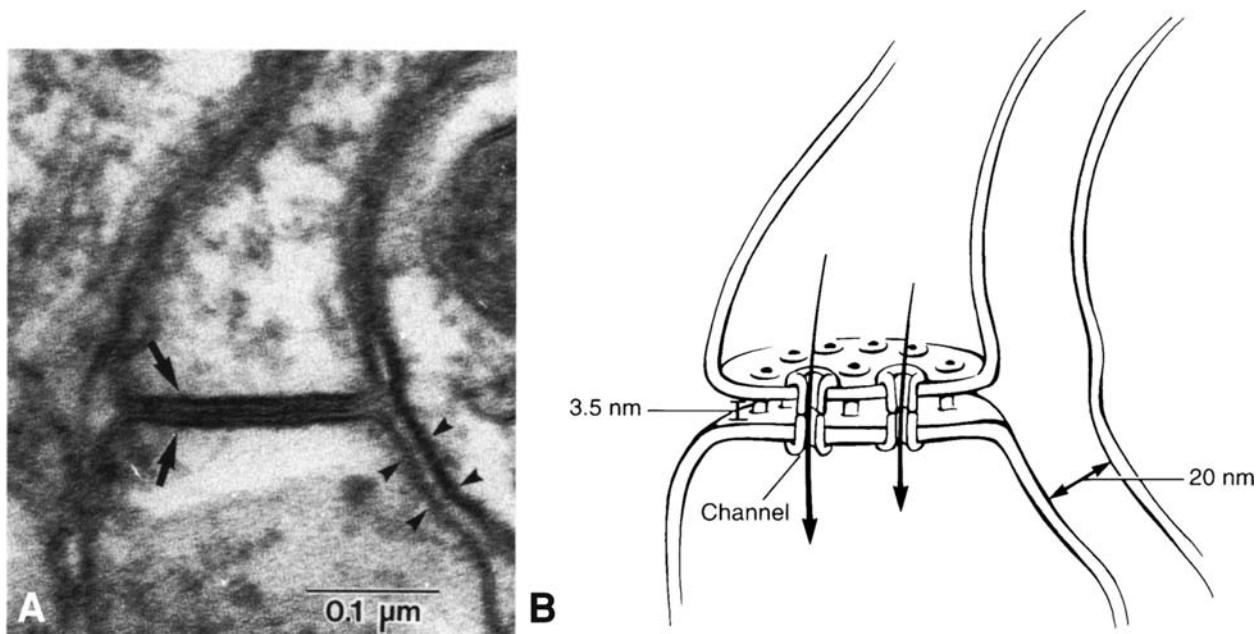


Fig. 8. Structure of the gap junction. (A) In the electron micrograph, the membranes of two glial cell processes in close apposition form a gap junction (arrows). Notice the wider extrajunctional space (arrowheads) to the right of the gap junction. (B) The diagram shows a section of the half channels in each membrane that join to form a pore, which allows communication between the cytoplasm of the two cells. The space between the two membranes of the gap junction is only 3.5 nm, much smaller than the extrajunctional space of 20 nm.

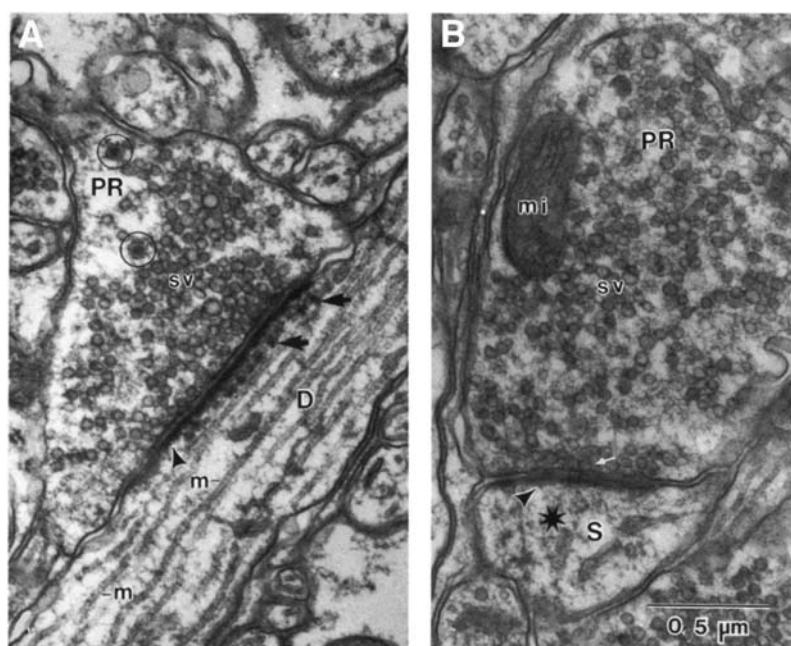


Fig. 9. Basic features of synaptic junctions in the central nervous system. (A) The presynaptic terminal (PR) ends on a dendrite (D) characterized by microtubules (m), and (B) another that ends on a spine (S) characterized by an actin filament network (asterisk). In both cases, synaptic vesicles (sv) are seen in the presynaptic terminal, and a prominent postsynaptic density (arrowhead) is located behind the postsynaptic membrane. In (A), the presynaptic terminal also contains dense-cored vesicles (circled), and in (B), the terminal contains a mitochondrion (mi). Some of the synaptic vesicles (white arrow) in (B) are located or docked near the presynaptic membrane. In the dendrite in (A), specializations called subsynaptic bodies (black arrows) are located on the cytoplasmic face of the postsynaptic density.

channels that connect the cytoplasm of the two cells while isolating the flow from the intracellular space. In neurons, these electrical synapses permit rapid and direct electrical transmission and may also play a role in synchronizing neuronal activity. However, their invariant form and paucity of regulatory molecules preclude any major involvement in plastic events. Gap junctions are also found elsewhere in the nervous system between supportive cells, called astrocytes, where they participate in buffering the extracellular ionic milieu.

Chemical synapses are the main sites of interactions of nerve cells (Fig. 9). All synaptic junctions share common features that guarantee precise and directed transmission of signals. Although the general scheme remains relatively constant, variability in some of the molecular components, such as neurotransmitter, ion channels, receptors, and second messengers, and the dynamic properties of the supporting cytoskeleton enable each synapse to maintain its individuality, record past experiences, and vary responses to new signals.

4.1. Synaptic Structure Follows a Basic Plan

Chemical synapses conform to a basic architectural plan despite their location along the neuron or within the nervous system itself, but during the past two decades, it has become apparent that the morphology of synaptic connections in the adult mammalian brain is not static. Synapses display structural plasticity, undergoing alterations in size,

shape, and number. These changes have important implications in synaptic transmission because they may modify the way in which incoming signals are received. Because morphologic alterations may reflect marked rearrangements of the molecular structure of synapses, these changes may be long lasting and signify the generation or maintenance of long-term processes, such as memory.

All synaptic junctions consist of presynaptic and postsynaptic elements (Fig. 9). In synapses in the central nervous system, very fine filamentous material extends between the two processes in the cleft region. The most conspicuous feature of the presynaptic terminal are the small (40 nm in diameter), clear synaptic vesicles containing acetylcholine or amino acid neurotransmitters. Mitochondria are also visible, as are dense-cored vesicles, some containing neuropeptides imported from the perikaryon and others containing catecholamines synthesized in the terminal. The portion of presynaptic membrane directly apposing the postsynaptic membrane is called the *active zone*. Sometimes, this area is marked by a dense submembranous array. The presynaptic nerve terminal contacts a postsynaptic element. In the central nervous system, this may be a cell body, dendrite (Fig. 9A), dendritic spine (Fig. 9B), another axon, or axon terminal.

The most striking postsynaptic feature is the dense, submembrane filamentous array beneath the postsynaptic membrane called the PSD (Fig. 9). Extending from this area is a fine meshwork of actin filaments

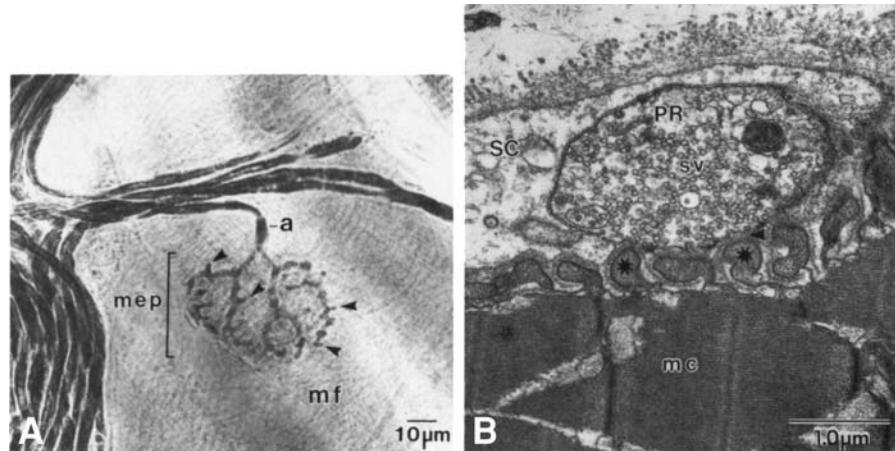


Fig. 10. Neuromuscular junction seen by (A) light and (B) electron microscopy. In (A), an axon (a) gives rise to a motor end plate (mep) on the muscle fiber (mf). The motor end plate exhibits many swellings (arrowheads). These represent the presynaptic terminals, one of which is seen at higher magnification in (B). The presynaptic terminal (PR), filled with synaptic vesicles (sv), contacts the sarcolemma folds (asterisks) of the muscle cell (me). A basal lamina (arrowhead) is found in between the terminal and sarcolemma. A portion of glial cell, known as a Schwann cell (SC), surrounds the terminal. (Part B courtesy of Virginia Kriho, University of Illinois at Chicago.)

and their binding proteins. In dendrites, this network is contiguous with microtubules; in dendritic spines, the filamentous web fills the head and neck region of this process. Some postsynaptic membranes lack a well-developed PSD.

Synapses are also found between nerves and muscles (Fig. 10). With the light microscope, axons can be seen to approach the muscle. At a specific site on the muscle, called the *end-plate region*, the axons give rise to several branches that display multiple swellings, each of which represents a presynaptic terminal. Ultrastructural examination reveals that the nerve terminal is closely apposed to the sarcolemma of skeletal muscle with a basal lamina lying in between the two components. The sarcolemma is thrown into distinctive folds. Acetylcholine receptors are located on the portions of the membrane directly opposing the active zones. A dense microfilamentous network extends from the membrane on the cytoplasmic face of the postsynaptic membrane and holds the receptors in place.

4.2. The Presynaptic Nerve Terminal Is the Site of Transmitter Release

Information transfer between neurons occurs in a matter of milliseconds. During this time, action potentials speed along the axonal membrane at rates between 1 and 100 m/s to the presynaptic nerve terminal, where the frequency of their firing is translated into specific quantities of neurotransmitter release. The rapidity of signal conduction over long distances demands that the nerve be ready to respond to a barrage of incoming action potentials at all times. Although axonal transport replenishes the terminal with some needed molecules, organelles, and neuropeptide-containing vesicles, the rates of delivery are not fast enough to prepare the terminal with the small molecules (i.e., acetylcholine, amino acids, catecholamines) comprising the bulk of chemical messengers that mediate neurotransmission. Even fast anterograde transport can only convey membranous vesicles at a rate of 200 to 400 mm (~1 ft) each day. Consequently, the nerve terminal comes equipped with mechanisms for neurotransmitter synthesis, storage, and release and mechanisms for membrane recycling. Presynaptic terminals, however, lack the elaborate protein synthetic and packaging machinery found in the perikaryon. Even free polyribosomes are difficult to detect, although there is some evidence for presynaptic protein synthesis. Most of the synthetic enzymes and some membrane

proteins required to construct synaptic vesicles must be imported from the cell body.

Neurotransmitters are primarily released from synaptic vesicles, although there is compelling evidence for additional nonvesicular release of acetylcholine from a cytoplasmic pool. Knowledge that synaptic vesicles mediate neurotransmitter release comes from the important discovery in 1952 by Paul Fatt and Bernard Katz that acetylcholine is released from terminals at the neuromuscular junction in quanta. A relatively constant number (about 10,000) of neurotransmitter molecules is released simultaneously. Since that time, neuroscientists have been actively engaged in experiments supporting this finding. Electron microscopic studies of nerve terminals revealed the presence of synaptic vesicles. When these vesicles were isolated, they were shown to contain acetylcholine. It was then proposed that the number of transmitter molecules in each quanta is equivalent to the number of acetylcholine molecules in each vesicle.

Synaptic vesicles occupy precise locales within the terminal; they are clustered and then docked near the active zone (Fig. 9B). They appear to be held in place there by actin, which is connected to the vesicle by a neuron-specific protein, synapsin. However, vesicles that have already released transmitter must be replaced by those next in line, necessitating a transient depolymerization of actin filaments so that the vesicles are free to approach the membrane. Phosphorylation of synapsin releases vesicles from the cytoskeleton, permitting them to proceed to the docking site at the presynaptic membrane. Specific proteins within the vesicle membrane and presynaptic membrane interact to hold the vesicle in place. Vesicle fusion occurs so fast that it is thought to involve a conformational change in a specific protein—perhaps a change in a preassembled calcium-dependent pore from a closed to an open state. The extra vesicle membrane, now a part of the presynaptic membrane, is internalized by a clathrin-dependent mechanism and brought to an endosomal sac, where membrane proteins are sorted and new vesicles pinch off the cisternae. It is thought that there are two release pathways: one for clear vesicles and another for neurosecretory vesicles. The latter are not concentrated near the active zone and require a lower calcium concentration and a higher frequency of stimulation for release at other sites along the terminal membrane. Dense-cored vesicle release may represent a basal secretion, in contrast with the phasic release of clear vesicles.

4.3. The Postsynaptic Element Is the Site of Signal Transduction

Neurotransmitters bind to specific sites called receptors on the postsynaptic membrane. This interaction is the initial step in a cascade of events that transduce the chemical message into an intracellular signal that affects the behavior of the postsynaptic neuron. Neuronal responses to incoming signals may include immediate alterations in membrane permeability or more long-lasting modifications in synaptic or neuronal architecture, which may modify the nature of the postsynaptic response. Signal transduction pathways in neurons attain an extraordinary level of complexity, increasing the number of adaptive responses by logarithmic proportions.

4.3.1. POSTSYNAPTIC DENSITY

Beneath the postsynaptic membrane is a dense filamentous array, the PSD (Fig. 9). The intimacy of its association with the overlying membrane suggests that it restricts receptors at that site, similar to the way acetylcholine receptors are clustered at the sarcolemmal membrane of the neuromuscular junction by actin and its binding proteins. However, several properties of the PSD suggest a more dynamic role in nerve transmission. Although the PSD usually is a saucer-shaped structure, there are variations of this basic form, including differences in length, curvature, and the presence or absence of perforations. Moreover, quantitative electron microscopic analyses reveal that these parameters change in specific brain areas with various physiologic and behavioral inputs. The PSD

contains actin that, together with its binding proteins also found here, may mediate dynamic changes in shape. The major protein in cerebral cortex PSDs, for example, is the 51-kDa, autophosphorylatable, Ca^{2+} /calmodulin-dependent protein kinase II, which comprises 30% to 50% of this structure. Mutant mice lacking one of the isoforms of this enzyme are also deficient in their ability to produce *long-term potentiation* (LTP). LTP is an electrophysiologic correlate of memory; after a given input, a synapse gets stronger and retains this new strength for a long period. Another important protein at the PSD is PSD-95, which appears to regulate the expression and function of some receptors in a synapse-specific manner.

4.3.2. DENDRITIC SPINES

Approximately 100 years ago, Santiago Ramón Cajal wrote that cortical dendrites seem to “bristle with teeth.” He called these protuberances collateral spines, and it is only recently that we have gained some insight into their precise function. Dendritic spines are protrusions of the dendritic surface that receive synapses, almost all of which are excitatory. They consist of spine heads of various diameters that are connected to the parent dendrite by necks, which also vary in length and thickness (Fig. 11). The spine shape is often categorized as thin, stubby, or mushroom shaped. Ribosomes have been found at the base of the spine neck, possibly functioning in local protein synthesis. Cortical neurons have thousands of dendritic spines, each located every few micrometers along the dendritic shaft.

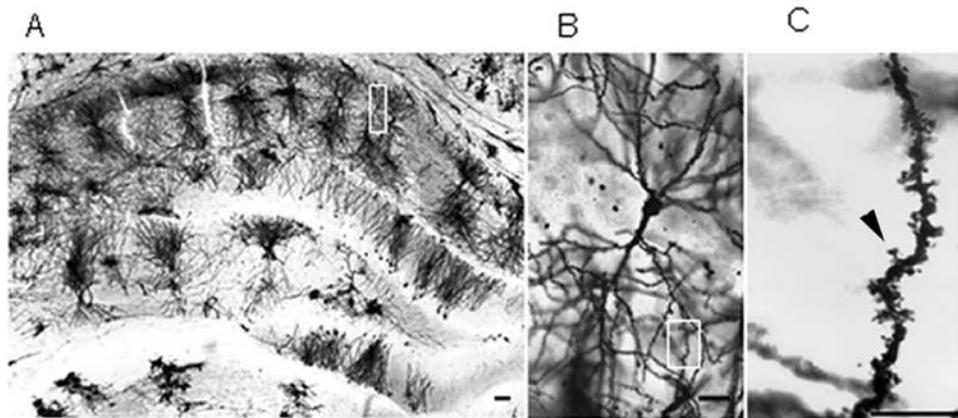


Fig. 11. Light micrographs of pyramidal cells and their processes in the hippocampus of an adult rat. **(A)** Pyramidal cells and their processes are seen at low magnification with Golgi-Cox staining. (scale bar = 100 mm). **(B)** High-magnification micrograph of the area delineated by the box in **(A)** shows the Golgi-impregnated cell body and dendrite of a neuron in the CA1 structure of the hippocampus (scale bar = 20 mm). **(C)** High-magnification micrograph of the area delineated by the box of a Golgi-impregnated dendrite in **(B)** shows dendritic spines, which appear as protuberances emanating from the dendrite. A spine with a long neck is indicated by the arrowhead. (scale bar = 10 mm). (Images courtesy of the laboratory of Dr. Subhash C. Pandey, Department of Psychiatry, University of Illinois at Chicago.)

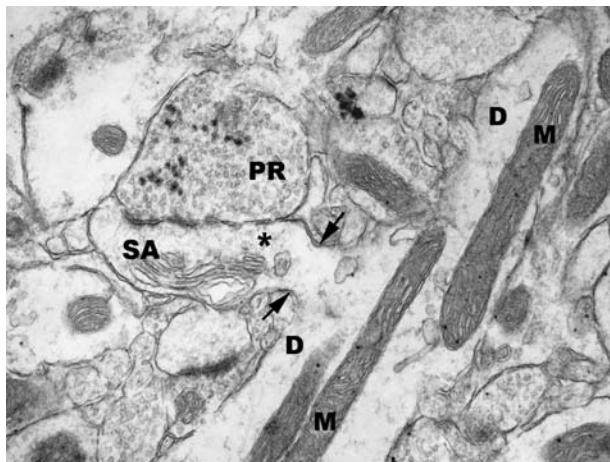


Fig. 12. Electron micrograph of a dendritic spine of the somatosensory cortex of an adult rat. A presynaptic terminal (PR) contacts a dendritic spine that emanates from a dendrite (D). The dendritic spine is characterized by a head (asterisk) and short neck (arrows) region. A membranous spine apparatus (SA) is seen in the head region. Mitochondria (M) are seen in the dendrite. (Image courtesy of Chiye Aoki, Center for Neural Science, New York University.)

The existence of dendritic spines greatly increases the surface area of a neuron and thus allows for a greater number of potential interactions with other neurons. In addition, research in recent years has revealed that the spines themselves can change shape according to changing physiologic conditions, such as altered hormone levels and environmental stress. One way in which synaptic inputs can change spine morphology is that the size of a spine is correlated with the numbers of docked vesicles in the presynaptic ending, thus suggesting the size of the spine head will be a monotonically increasing function of the amount of current entering the neuron through the spine. The cytoskeletal network comprising the interior of the spine includes actin and myosin. These proteins may play a role in spine contractility, which may lead to changes in the shape or number of spines. Some authors believe that abnormalities of dendritic spine morphology and chemistry could result in aberrant synaptic signaling accompanied by neurologic disorders such as mental retardation.

Dendritic spines may function as calcium isolation compartments that decouple calcium changes in the spine head from those in the dendritic shaft and from neighboring spines. This possibility has important implications for explaining LTP. LTP may be synapse specific; its expression is a function of activation of calcium-dependent processes at the synapse, such as the activity of the Ca^{2+} and calmodulin-dependent

protein kinase II, which is also implicated in long-term processes. Calcium levels may be regulated by the membranous *spine apparatus* (Fig. 12), because Ca^{2+} -ATPase, the IP receptor, and calcium are localized there.

5. GLIAL CELLS

Neuronal form is largely a function of the internal cytoskeletal framework. However, neurons are also supported externally by a second type of cell in the nervous system, the glial cell. Glial cells are present in the central and peripheral nervous systems. Glia fill in all spaces not occupied by neurons and blood vessels, surrounding and investing virtually all exposed surfaces in the central nervous system and axons in the peripheral nervous system. Glial cells vary in morphology, and their function is not restricted to mechanical support. In the central nervous system, glia are subdivided into four main types: *astrocytes*, *microglia*, *oligodendrocytes*, and *ependymal cells*. In the peripheral nervous system, *Schwann cells* function in a manner similar to that of oligodendrocytes, forming insulating *myelin sheaths* around axons that facilitate conduction.

5.1. Glia Play a Variety of Roles in the Nervous System

5.1.1. ASTROGLIA

Astrocytes are stellate-shaped cells with a multitude of processes that radiate from the cell (Fig. 13A). These processes are supported internally by a glial-specific intermediate filament protein, *glial fibrillary acidic protein*. *Protoplasmic astrocytes* are more abundant in gray matter and have relatively short, branching cytoplasmic processes; *fibrous astrocytes*, on the other hand, are usually seen in white matter and display fewer processes and less branching. Some astrocytic processes may terminate as swellings called *end-feet* on neurons and blood vessels (Fig. 13A, B). Astrocytes may accumulate extracellular potassium resulting from the repeated firing of neurons. The potassium may then be released by astrocytic end-feet onto blood vessels to increase their diameter. In this way, increased neuronal activity may be supported by a concomitant increase in blood flow and oxygen consumption.

Importantly, astrocyte end-feet contribute to the formation of the *blood-brain barrier* (Fig. 13B). Here, the end-feet are in close proximity to specialized capillary endothelial cells and their endothelial basal lamina. There are no *tight junctions* between glia cells.

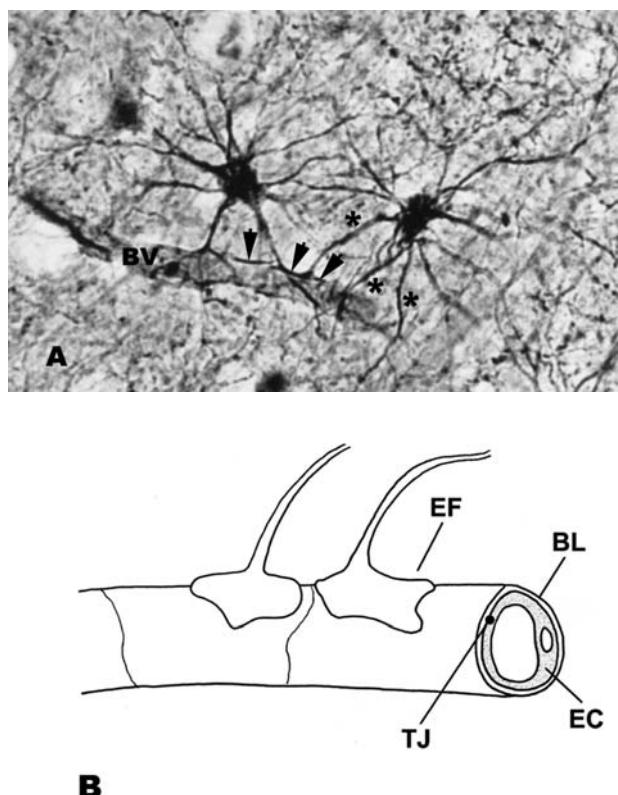


Fig. 13. Astrocytes from monkey cortex. The light micrograph in (A) shows a brain section treated with Cajal's silver stain, which stains internal fibrous elements black. Two star-shaped astrocytes are seen, and the processes emanating from them are labeled by asterisks. The astrocyte end-feet (arrows) emerging from the indicated processes are in contact with a blood vessel (BV). The diagram in (B) shows the end-feet (EF) in intimate contact with a capillary. At the right, in cross-section, the endothelial cell (EC) of the capillary is seen surrounded by a basal lamina (BL). A tight junction (TJ) is present between endothelial cells comprising the capillary. There are no tight junctions between astrocyte end-feet. A pericyte (not shown) surrounds the basal lamina.

However, the functional integrity of the tight junctions between *endothelial cells*, which prevent entry of solutes and fluid into the brain tissue, appears to depend upon normal functioning of the astrocyte. Active transport by specific receptor-mediated endocytosis permits entrance across the capillary wall. Some lipid-soluble molecules, such as steroid hormones, oxygen, and carbon dioxide, can pass through the barrier, but other molecules, such as amino acids, require specific carrier molecules.

Another blood-brain barrier is associated with the *choroid plexus epithelium*, located within the lateral, third and fourth ventricles; this is a highly vascularized epithelium, which produces cerebrospinal fluid. Tight junctions between epithelial cells constitute the

blood-brain barrier between blood and cerebrospinal fluid. The choroids plexus is a modification of the ependyma, a ciliated cellular lining of the central canal system of the brain and spinal cord.

Normally, the blood-brain barrier also excludes cells, such as leukocytes, immune mediators, as well as dyes and antibiotics from entering the brain. However, the blood-brain barrier can be breached, for example, by bacterial components, in several ways. One strategy is invasion engineered by the pathogen itself. For example, astrogli dysfunction caused by the bacterium *Listeria* results in an opening of tight junctions and subsequent penetration by this organism. The spirochete *Treponema* can cross between blood-brain barrier endothelial cells. The two pathogenic microorganisms, *Streptococcus pneumoniae* and *Plasmodium*, have specific receptors on endothelial cells that permit transmigration across the barrier.

The blood-brain barrier is not just a “gate” to prevent entry into the blood, but rather a mode of communication between the nervous and immune systems. The endothelial or epithelial barriers themselves can secrete *cytokines*, signaling proteins and peptides, especially important in immune responses, and also be regulated by cytokines. Some regions of the brain, such as the *circumventricular organs*, have leaky vessels and lack a blood-brain barrier; most epithelial regions of the ependyma (except the choroid plexus described above) are also leaky. Because of the restrictions on the size and chemical composition of molecules that are able to cross the blood-brain barrier, drug delivery to the brain is hindered, thereby presenting a challenge to the development of pharmaceuticals capable of crossing the barrier.

As stated earlier, glial cells formerly were theoretically assigned modest roles in nervous system function, being thought of primarily as supportive in ways both mechanical and metabolic. Now, however, it is understood that astrocytes can play dynamic roles at some of the most important synapses in the nervous system. Philip Haydon has given voice to this fact in his concept of the “tripartite synapse” that includes not only the classically recognized presynaptic and postsynaptic elements but also an enveloping glial cell. For example, in synapses that use glutamate as the transmitter—glutamate being the oldest and most rapidly signaling excitatory transmitter—the glutamate released is actively transported into a neighboring astrocyte thus limiting its duration of action in the synapse. In the astrocyte, it is enzymatically transformed into glutamine and then released. The presynaptic glutamatergic ending actively transports it into

the nerve cell and, using the enzyme glutaminase, makes more glutamate. Thus, changes in the morphology and biochemical efficiency of the astrocytes in question would have important neuromodulatory consequences.

Morphologic analyses indicate that astrocytic processes preferentially contact neuronal surfaces over those of other glia, despite a ratio of glia to neurons of at least 10 to 1. Other evidence suggests that associations between neurons and glia are constantly in flux throughout the life of the organism. Glia may promote or inhibit the outgrowth of neuronal processes during development by synthesizing and secreting various adhesion molecules. In some parts of the developing nervous system, such as the cerebellum and neural tube, radial glial cells form a transient scaffold that guides the migration of immature neurons to their final destinations. The migrating neurons wrap around these pole-shaped cells and crawl along them. After completion of the trip, the radial glia disappear and may be transformed into astrocytes. In the central nervous system, astrocytes and macrophages, called microglia, remove the cellular debris resulting from degenerative processes.

5.1.2. MICROGLIA

Microglia constitute only a small portion (~5%) of the glial cell population but are roughly equivalent in number to neurons (~15% of the cell population). They originate from bone marrow monocyte precursor cells and are, therefore, thought to be part of the *mononuclear phagocyte system*. Along with astroglia, microglia are part of the immune system of the CNS. They are the smallest of the glial cells. Embryonically, microglia are ameboid and in the adult, resting state display short processes, branched processes. At sites of injury and disease, microglia proliferate, return to their ameboid configuration, and become motile and actively phagocytic. Cell surface markers central to immune function, such as the MHC class II molecules, are constitutively expressed on adult, resting microglia. However, when activated, a large number of receptor types are rapidly upregulated, and a number of secretory products, such as *cytokines* and *chemokines*, are produced that can either defend or damage the diseased brain. Microglial generation of free radicals (e.g., reactive oxygen or nitrogen intermediates) are also implicated in defense of, as well as damage to, neurons. Other secretory products play a role in blood-brain barrier breakdown, which may result in leukocyte infiltration into the nervous system and tissue destruction. In addition to these inherent features of microglia, these cells can also recruit

lymphocytes, monocytes, and neutrophils, which, in turn, can also act in defense of the brain. Microglia have been implicated in a wide spectrum of diseases, including those caused by microorganisms, such as human immunodeficiency virus-associated dementia, cytomegalovirus, herpes simplex virus, cerebral malaria, as well as neuroinflammatory and neurodegenerative diseases, such as multiple sclerosis, Alzheimer's disease, Parkinson's disease, and Huntington disease.

Microglia are not the only immune cells in the CNS. *Mast cells*, which are also derived from bone marrow and function in the immune system, occur in the healthy and diseased adult brain. In experimental allergic encephalitis, a mouse model of multiple sclerosis, mast cells are seen close to plaques, characteristic of this disease. Mature mast cells have granules that function as storage for effector molecules, including histamine and neuropeptides, and enzymes. Upon activation, these cells synthesize and release various molecules, including those involved in immune reactions, such as cytokines; they also take up molecules and particles, such as bacteria. Interestingly, mast cell number in the brain is a function of reproductive behavior and endocrine status and, therefore, may be involved in neuroimmune function.

5.2. GLIA FORM MYELIN SHEATHS THAT INCREASE THE SPEED AND EFFICIENCY OF CONDUCTION IN AXONS

Oligodendrocytes and Schwann cells form myelin sheaths around axons. These sheaths are formed by the attenuation of the glial cytoplasm to such an extent that most of the sheath is composed of concentric layers of plasma membrane wrapping around the axon (Fig. 14 and Fig. 15). In oligodendrocytes, several processes extend out from the cell, tapering as they encounter an axon, and wrap around a portion of its length. One oligodendrocyte can ensheathe many axons, all of different neuronal origins. Individual Schwann cells dedicate themselves to a single axon. The exposed patch of axon in between adjacent segments of the myelin sheath is called the *node of Ranvier* (Fig. 14 and Fig. 15). Most of the Na^+ ion channels of the axon are confined to this site. Because of the lack of channels between the nodes and great insulating action of the myelin sheath, there is virtually no current flow across these segments. The action potential bypasses these stretches of membrane by jumping from node to node. This type of rapid propagation is known as *saltatory conduction*. An added advantage is that energy is conserved; fewer ions enter and leave the axon so less energy is

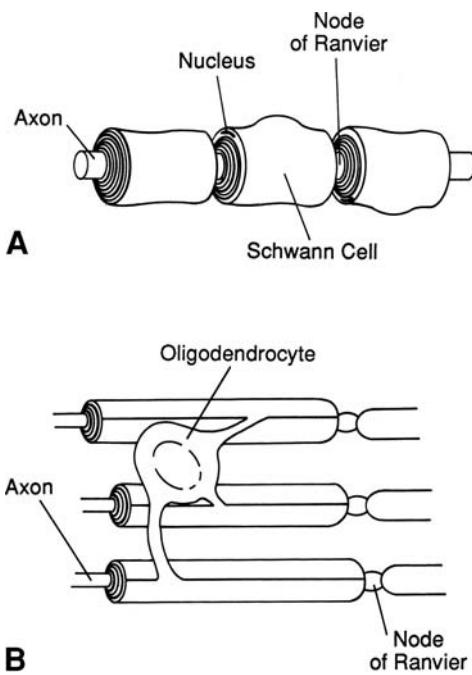


Fig. 14. Diagrams of myelinated axons of the peripheral and central nervous systems. (A) In the peripheral axon, several Schwann cells wrap their plasma membrane concentrically around a single axon. The stretch of axonal membrane, called the axolemma, between adjacent Schwann cells is known as the node of Ranvier. (B) In the central axon, several glial processes emerge from one oligodendrocyte and ensheath several axons of different origins.

expended in returning the membrane to its original polarized state by active transport mechanisms.

5.3. Neuropathologies Related to Myelin

There are many neuropathologies related to myelin that have various etiologies; some are acquired and others are inherited. An example of the former is multiple sclerosis, an inflammatory, demyelinating disease, characterized by muscle weakness and subsequent problems, such as declining mobility, extreme fatigue, and impaired coordination and speech. An animal model of multiple sclerosis that exploits the antigenicity of myelin proteins and subsequent robust immune response is *experimental allergic encephalitis*. One class of these proteins, *myelin basic proteins* (MBP), plays a key role in myelin compaction. Related proteins are generated from a single MBP gene by alternative RNA splicing. Another animal model of this disease is the *shiverer mutant mouse*. These mice also exhibit demyelination, display tremors, convulsions, and die young. They lack five of the six exons for the myelin basic protein gene and exhibit only 10% of

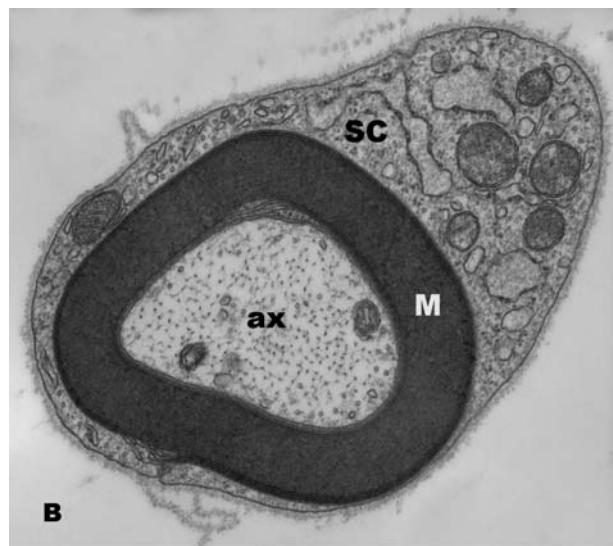
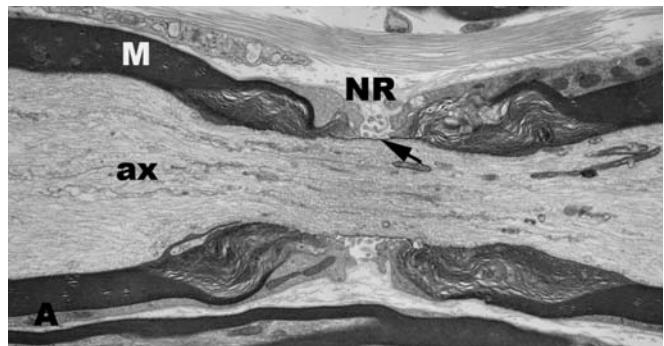


Fig. 15. Electron micrographs of myelin sheaths of axons. (A) A longitudinal section through the axon (ax) shows that the sheath is interrupted at regular intervals, called nodes of Ranvier (NR), where portions of the axonal membrane, called the axolemma (arrow), are exposed. Sodium ion channels are concentrated in the axolemma at the nodes. (B) A cross section of the axon (ax) shows the surrounding myelin sheath (M) and Schwann cell (SC) cytoplasm. (Images courtesy of Thomas Deerinck and Mark Ellisman, the National Center for Microscopy and Imaging Research.)

the MBP present in wild-type mice. Injection of the wild-type gene into the fertilized eggs of the *shiverer* mouse by transgenic technology rescues the mutant, which then can express 20% of the normal levels of MBP; however, apart from some tremors, these mice do not have convulsions and live a normal life span.

In *Guillain-Barré syndrome (acute inflammatory demyelinating polyradiculoneuropathy)*, an autoimmune disease of the peripheral nervous system, there is a large accumulation of lymphocytes, macrophages, and plasma cells around nerve fibers

within nerve fascicles. Large portions of the myelin sheath are damaged. These symptoms are consistent with a T-cell-mediated immune response directed against myelin. Symptoms include muscle paralysis and loss of muscle coordination and cutaneous sensation.

Another demyelinating disease is hereditary motor and sensory neuropathy, or *Charcot-Marie-Tooth syndrome*. Here, there is an increased production of *peripheral myelin protein PMP22*, originally due to a duplication of a region of DNA. Peripheral neuropathy results, as well as cycles of myelination and demyelination, resulting in reduced motor nerve conduction; patients present with impaired gait.

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Bruce W. Newton

CONTENTS

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1. INTRODUCTION

The central nervous system (CNS) is divided into a rostral *brain* and a caudal *spinal cord*. The brain is contained in the cranial cavity, and the spinal cord is located in the vertebral canal that is formed by the 31 vertebral foraminae from the individual vertebra. The two are continuous with one another at the foramen magnum of the occipital bone. Any neural structure that lies outside the pia mater covering of the CNS is considered part of the peripheral nervous system. Therefore, the 12 pairs of cranial nerves, which arise from the brain, and the 31 pairs of spinal nerves originating from the spinal cord with their associated ganglia are, by convention, part of the peripheral nervous system. Both the brain and spinal cord are organized into *gray matter*, where the neuronal cell bodies are located and *white matter*, which contains the long myelinated tracts of the CNS. Spinal cord gray matter is centrally located and surrounded by white matter, whereas the opposite occurs in the cerebral cortex.

2. SPINAL CORD EXTERNAL ANATOMY

The spinal cord, continuous with the brain's medulla oblongata, is a long cylinder beginning at the foramen magnum and extending, in adults, to the intervertebral disk between the first and second lumbar vertebrae. It is divided into 31 repeating

segments composed of 8 cervical, 12 thoracic, 5 lumbar, 5 sacral, and 1 coccygeal segment. Each spinal cord segment has an associated pair of spinal nerves that originate as a series of fine rootlets. Each spinal nerve is formed from *dorsal root fibers*, which are sensory axons whose cell bodies reside in *dorsal root ganglia* located outside the CNS, and *ventral root fibers*, which are motor axons originating from ventral horn motor neurons in the spinal cord gray matter (Fig. 1, Fig. 2, and Fig. 3). At spinal cervical levels C1 through C4, axons forming the accessory nerve (cranial nerve [CN] XI) originate from the lateral side of the spinal cord intermediate between the dorsal and ventral root fibers before ascending and entering the skull via the foramen magnum. In contrast with the number of spinal cord segments, the vertebral column, which surrounds and protects the spinal cord, has 7 cervical vertebrae, 12 thoracic vertebrae, 5 lumbar vertebrae, 5 sacral vertebrae typically fused into a single sacrum, and 3 to 4 coccygeal vertebrae fused into a common coccyx. During development, the nascent embryonic spinal cord extends the length of the vertebral column, but subsequent *in utero* differential growth of the vertebral column versus the spinal cord results in the infant spinal cord ending at the caudal aspect of the third lumbar vertebra. Additional growth results in the adult spinal cord ending at the level of the intervertebral disk between the first and second lumbar vertebrae. The remainder of the vertebral canal below the level of the second lumbar vertebra contains the obliquely oriented dorsal and ventral roots traveling to their proper point of exit from the vertebral canal at the appropriate

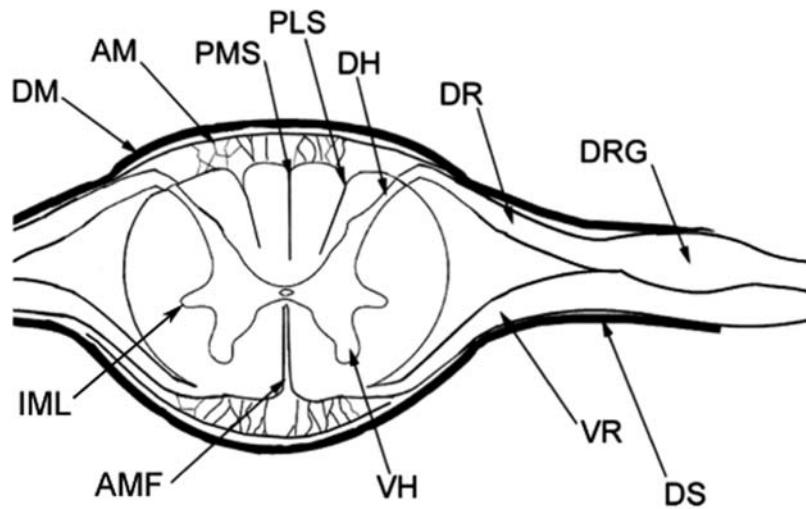


Fig. 1. Schematic diagram of a cross-section of the spinal cord. The gray matter occupies the central region of the spinal cord. The ventral horn (VH) and dorsal horn (DH) are found in all spinal segments; the intermediolateral gray horn (IML) is found in the T1 through L2 segments. The anterior median fissure (AMF) extends through the white matter to the gray matter. The posterior median sulcus (PMS) and posterolateral sulcus (PLS) are located on the posterior side of the white matter. Dorsal root (DR) axons that enter the dorsal horn originate from cell bodies in the dorsal root ganglion (DRG). The ventral root (VR) is composed of axons from motor neurons in the ventral horn. The dura mater (DM) covers the spinal cord and extends to the intervertebral foramen as a dural sleeve (DS) that merges with the epineurium. The arachnoid membrane (AM) lies deep to the dura mater and has fibrous strands that extend to the pia mater on the surface of the spinal cord.

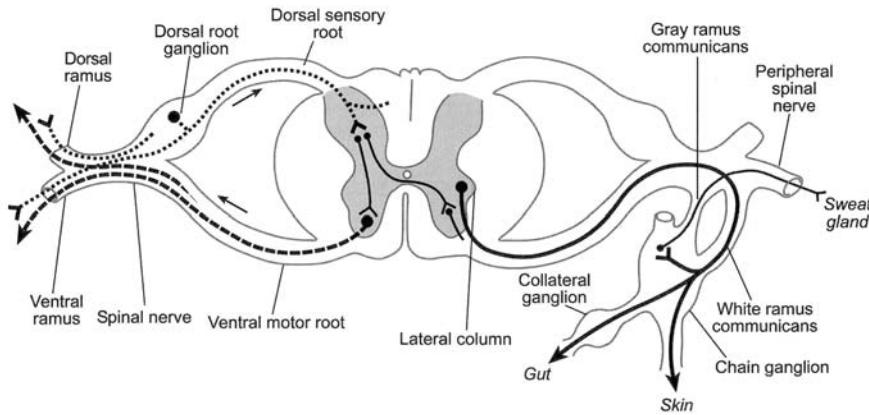


Fig. 2. Components of spinal nerves. A typical somatic motor and sensory pathway of a spinal nerve is shown on the left; the final common sympathetic nervous system pathway is shown on the right. On the left, somatic and visceral sensory axons (*small dashes*) travel through the dorsal or ventral rami and the spinal nerve before reaching their pseudounipolar cell body of origin in the dorsal root ganglion. The axons then form the dorsal sensory roots and enter the dorsal gray horn or dorsal white matter. Axons of somatic motor neurons (*large dashes*), located in the ventral gray horn, leave the CNS to form the ventral motor roots. The motor axons then enter the spinal nerve and diverge into a dorsal or ventral ramus to travel to their skeletal muscle target. On the right, preganglionic sympathetic axons (*heavy solid line*) leave the CNS through the ventral motor roots. The axons then enter the spinal nerve and travel through a white ramus communicans to enter the chain ganglion. From there, preganglionic sympathetic axons pass out of the chain ganglion via a variety of routes (*heavy lines with arrows*) to reach the postsynaptic sympathetic neurons (not shown). Some preganglionic sympathetic axons synapse on postganglionic sympathetic neurons in the chain ganglion. If so, the postganglionic axon (*light solid line*) leaves the chain ganglion through the gray rami communicans to enter a ventral or dorsal ramus to reach its peripheral target. Connections for spinal reflexes are shown in the gray matter and are discussed in the chapter of this volume dealing with the organization of the spinal cord.

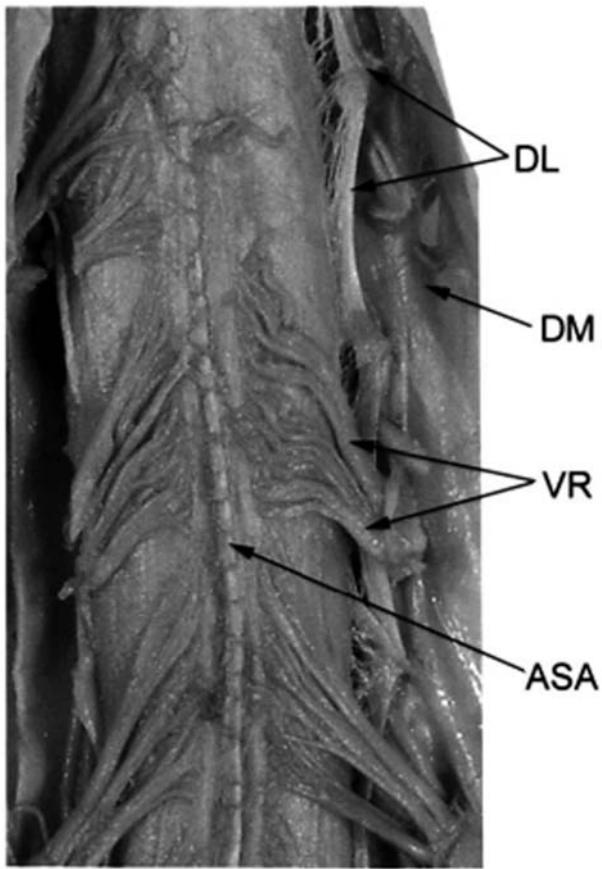


Fig. 3. Anterior surface of the spinal cord. The dura mater (DM) has been cut to reveal the ventral rootlets (VR) that comprise one ventral root of the spinal cord. The anterior spinal artery (ASA) lies in the anterior median fissure. The ventral roots have been removed from the right side of one segment to reveal the denticulate ligament (DL), which is a tooth-like pia mater extension from the surface of the spinal cord to the dura mater.

intervertebral foramina. This collection of dorsal and ventral roots, contained within the lumbar cistern of the dural sac, is called the *cauda equina* because of its resemblance to a horses' tail.

The spinal cord has two noticeable swellings along its length: a *cervical enlargement* at lower cervical to upper thoracic levels (C4 through T1) and a *lumbar enlargement* at lumbar to upper sacral levels (L1 through S1). These swellings are formed by the larger number of motor and sensory neurons needed for the innervation of the upper and lower extremities compared with the thoracic, abdominal, and sacral regions. At its termination, the spinal cord narrows into the *conus medullaris*, representing a reduction in neuronal cell bodies and myelinated tracts.

The spinal cord's anterior (ventral) surface has a deep *anterior median fissure* along its entire length, which typically contains the anterior spinal artery (Fig. 1 and Fig. 3). The anterior median fissure extends deeply into the spinal cord, dividing the anterior half of the spinal cord into two separate cylinders. On its posterior (dorsal) side, several longitudinal depressions are visible: a midline *posterior median sulcus* and two *posterior lateral sulci* on either side of the posterior median sulcus. The dorsal roots of the individual spinal nerves originate from the posterolateral sulcus. A *posterior intermediate sulcus*, intermediate between the dorsal median sulcus and the posterolateral sulcus, is present beginning at the upper thoracic and cervical spinal levels. Its formation results from the location of two separate ascending sensory tracts, the *fasciculus gracilis* and *fasciculus cuneatus*, from the lower and upper limbs, respectively (Fig. 1 and Fig. 4).

2.1. Spinal Cord Internal Anatomy

The spinal cord is divided into an outer layer of white matter and an inner core of gray matter (Fig. 1 and Fig. 4). The *white matter* is composed of longitudinally oriented myelinated sensory and motor fiber tracts that ascend and descend the length of the spinal cord (Fig. 4). The *gray matter* takes the shape of an "H" and is composed of paired dorsal and ventral gray horns that run the length of the spinal cord. From the first thoracic to the second lumbar spinal segments, the gray matter contains a small additional horn (cell column), known as the intermediolateral cell column, intermediate between the ventral horn and dorsal horn. Contained within the horizontal, interconnecting gray matter is the *central canal*, the part of the ventricular system of the CNS that is most representative of the embryonic neurocyst of the neural tube.

2.1.1. SPINAL GRAY MATTER ANATOMY

According to the cytoarchitecture, the spinal gray matter is divided into 10 regions (I through X) as described by Rexed. The dorsal gray horns are sensory in nature and receive somatic and visceral afferent inputs. The ventral gray horns have a motor function and contain the motor neurons whose axons innervate skeletal muscles. The motor neurons are arranged in columns oriented in a rostrocaudal fashion with each column innervating a skeletal muscle or group of functionally related skeletal muscles. For example, motor neurons that innervate the quadriceps femoris muscles of the anterior thigh are

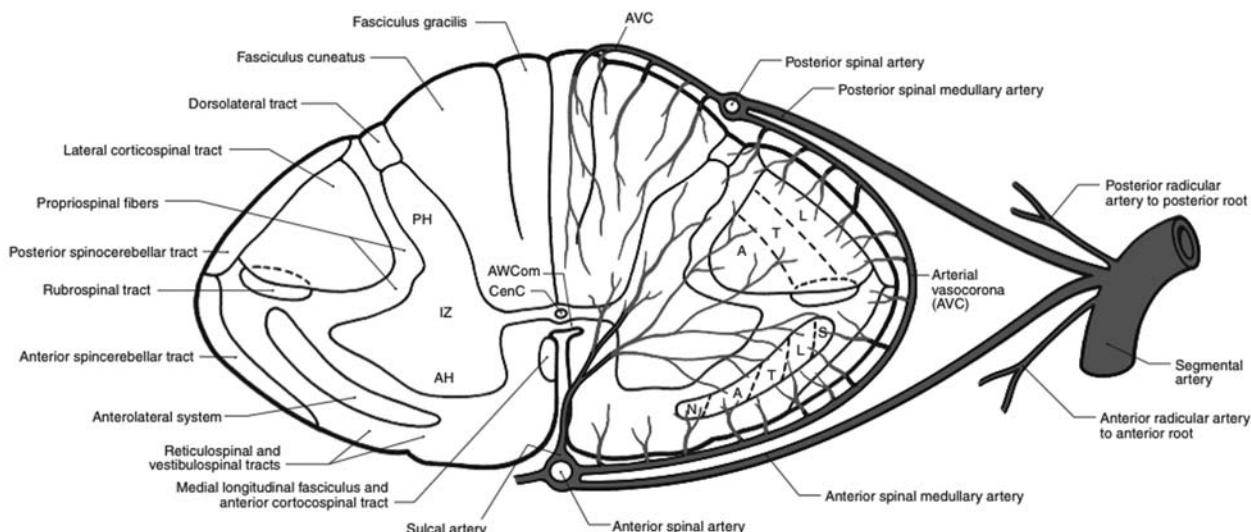


Fig. 4. The arterial supply (right side) and internal anatomy of the spinal cord. Both the anterior and posterior spinal arteries receive contributions from anterior and posterior spinal medullary arteries that arise from segmental arteries. An arterial vasocorona (AVC) creates anastomoses between the anterior and posterior spinal arteries. The gray matter has a posterior horn (PH; also called dorsal horn), an anterior horn (AH; also called ventral horn), and an intermediate zone (IZ). The central canal (CenC) and the anterior white commissure (AWCom) are labeled. The major white matter tracts are named. The somatotopic arrangement of axons within two tracts is seen on the right side of the schematic: N, neck; A, arm; T, trunk; L, leg; S, sacral region. (Used with permission, from *Neuroanatomy An Atlas of Structures, Sections, and Systems*, 7th ed., by Duane Haines, New York: Lippincott Williams and Wilkins.)

located in the L3 and L4 spinal cord segments. Within this column, individual clusters of motor neurons innervate just one of the four muscles comprising the quadriceps femoris. Motor neuron columns exhibit a rostrocaudal somatotopy so that as we descend in the spinal cord, the motor neuron columns innervate muscles located successively lower in the body. There is also a mediolateral somatotopy, such that motor neurons innervating distal limb musculature are more lateral than those motor neurons innervating more proximal limb muscles. Superimposed on this mediolateral arrangement, motor neurons that innervate limb flexors lie dorsal to those that innervate extensor muscles.

2.1.2. THE SPINAL AUTONOMIC NERVOUS SYSTEM

A portion of the intermediate region of the spinal gray matter (Rexed laminae VII and X) contains *preganglionic* (also called presynaptic) neurons of the *autonomic nervous system* (ANS). The ANS has two divisions: the *sympathetic nervous system* (SNS) and the *parasympathetic nervous system* (PSNS). Both the SNS and PSNS use a two-neuron chain as the final common pathway to innervate their peripheral targets: smooth muscle, cardiac muscle, and glands. (Note: The ANS never innervates striated muscle fibers.) The ANS neuronal cell body located in the CNS is called *preganglionic* and the neuronal

cell body located in the periphery is called *postganglionic* (also called postsynaptic). All preganglionic SNS neurons are found in thoracolumbar spinal cord segments T1 through L2. The parasympathetic division, as the name suggests, lies on either side of the SNS. The preganglionic PSNS neurons are located in craniosacral positions and are associated with cranial nerves III, VII, IX, and X and sacral spinal cord segments S2 through S4.

The majority of SNS preganglionic neurons are found in the intermediolateral cell column while approximately 15% are distributed more medially in a ladder-like pattern that extends through lamina VII to lamina X dorsal to the central canal. This more medial distribution of preganglionic SNS neurons is of clinical significance because syringomyelia (a cavitation of the spinal cord that starts adjacent to the central canal) that extends into the thoracic or upper lumbar spinal segments can destroy these medially located preganglionic SNS neurons and compromise ANS function.

Preganglionic SNS axons exit the CNS via the ventral roots. After entering the spinal nerve, the axon enters the ventral primary ramus and then passes through the *white ramus communicans* found on ventral primary rami T1 through L2 (Fig. 2). At this point the preganglionic axon can do one of several things. First, it can synapse on postganglionic

SNS neurons located in *sympathetic chain ganglia* adjacent to the ventral primary ramus where the preganglionic SNS axon left the CNS. Then the postganglionic SNS axon reenters the ventral primary ramus through a *gray ramus communicans* (found on all ventral primary rami) to innervate sweat glands, arrector pili muscles (i.e., smooth muscle fibers attached to the base of hairs), and vascular smooth muscle in skeletal muscles and skin. Second, some preganglionic SNS axons, once inside the sympathetic chain, can ascend or descend in the sympathetic chain, which extends from the base of the skull to the coccyx, before synapsing on postganglionic SNS neurons within the sympathetic chain ganglia whose axons innervate the head and the upper and lower extremities. The postganglionic SNS axon leaves the sympathetic chain via a gray ramus communicans before entering the spinal nerve for peripheral distribution. Those postganglionic SNS axons destined for the head leave the superior cervical ganglion of the sympathetic chain via the carotid nerve. Finally, other preganglionic SNS neurons, whose function is the control of viscera, send axons into the sympathetic chain. These preganglionic axons leave the sympathetic chain, without synapsing, and form cardiac or splanchnic nerves that synapse on SNS postganglionic neurons found in SNS ganglia that lie on the aorta or viscera. In humans, the ratio of preganglionic to postganglionic SNS neurons is more than 1:100 indicating the divergence of the CNS command to the periphery. This helps to explain why SNS actions are widespread in the body, whereas the PSNS control of viscera is precisely controlled because fewer postganglionic PSNS neurons are controlled by a single preganglionic PSNS neuron.

Parasympathetic preganglionic neurons reside on the white-gray border of lamina VII in spinal segments S2 through S4. Unlike the T1 through L2 spinal segments, there is no discernible intermediolateral cell column. Preganglionic PSNS axons leave the spinal cord via the ventral roots and enter the S2 through S4 ventral primary rami of the lumbosacral plexus. Once inside the pelvis, the axons leave the ventral primary rami as *pelvic splanchnic nerves* that synapse on postganglionic PSNS neurons found next to or within pelvic organs, erectile tissue, and hindgut (i.e., descending and sigmoid colon, rectum, and anal canal).

Just as there are sharp, precisely localized somatic sensations arising from skin, skeletal muscle, and bone, there are dull, diffusely localized visceral sensations arising from the internal organs. Visceral

sensations are relayed to laminae VII and X in the T1 through L2 and S2 through S4 spinal segments via the axons of dorsal root ganglion neurons. These visceral afferent axons contain a wide variety of peptidergic neurotransmitters that synapse on, and demarcate the position of, the preganglionic SNS and PSNS neurons. Visceral sensations also enter the CNS via CN IX and X and carry afferent information from the carotid body and sinus, the thorax, foregut, and midgut.

2.1.3. SPINAL CORD SEXUAL DIMORPHISM

The lumbosacral spinal segments have been found to be *sexually dimorphic* (i.e., to differ between the sexes). Sex differences in afferent input and neuron numbers appear to be restricted to the reproductive organs and their associated musculature. The numbers of motor neurons that innervate the muscles surrounding erectile tissue are more numerous in males than in females, as these muscles are larger in males. These neurons, in either sex, are found in the S2 through S4 spinal segments in *Onuf's nucleus*. Males also have greater numbers of L1 and L2 motor neurons that innervate the cremaster muscle. This muscle surrounds the testes and raises and lowers them in response to changes in temperature. The female cremaster muscle, much smaller in size, surrounds the round ligament of the uterus as it passes through the inguinal canal and enters the labia majora.

Although not yet shown for humans, male rats have a larger number of lumbar preganglionic SNS neurons for the control of reproductive organs. Also in rats, there is a dramatic sexual dimorphism for the number of peptidergic afferents in laminae VII and X surrounding lumbar SNS and sacral PSNS preganglionic neurons with males having larger numbers of these peptide-containing afferents than do females. For at least one of these peptides, galanin, its amount rises and falls with the rat's estrous cycle. Rats also display a sexual dimorphism for spinothalamic neurons in lumbar laminae VII and X. Males have more of these peptidergic, somatic and visceral sensation relaying neurons than do females. Not all sexual dimorphisms favor males having greater numbers of neurons or afferent inputs. There is a population of laminae VII and X neurons in the female lumbar spinal cord that produces dynorphin, a pain-suppressing peptide, just before and during parturition. Male rats lack this population of dynorphin-producing neurons. A similar population of lumbar pain-suppressing neurons may be present in human females, as the ability to withstand pain increases dramatically starting about 11 days before birth.

In all the instances mentioned in this section, the establishment of sex differences are under the control of the hormonal milieu present during the perinatal period. The sex differences in afferent input and spinothalamic neuron number may have future clinical relevance, as many of the peptidergic neurotransmitters contained in these structures are involved in suppressing nociception (i.e., pain). Because there are known sex differences in the human response to noxious stimuli, pain-suppressing pharmaceuticals can be developed that take into account the inherent sex differences in spinal neuropeptides that are under the control of gonadal hormones whose titers vary throughout life.

2.2. Blood Supply of the Spinal Cord

The spinal cord receives its blood supply from three longitudinal arteries, which are supplemented from segmental vessels along the length of the spinal cord (Fig. 3 and Fig. 4). Extensive anastomoses occur between the longitudinal arteries and the segmental arteries. The *anterior spinal artery* is the principal artery of the anterior two-thirds of each spinal cord segment. As such, it gives off numerous sulcal branches (also called central branches) that course within the anterior median fissure to enter the spinal cord. The smaller, paired *posterior spinal arteries* are responsible for the remaining posterior third of the spinal cord, mainly the dorsal columns. The anterior spinal artery originates as a common trunk from the union of the paired vertebral arteries as they pass through the foramen magnum into the cranial cavity. The posterior spinal arteries arise either from the vertebral arteries or the posterior inferior cerebellar arteries, which are typically branches of the vertebral arteries. The anterior spinal artery runs the entire length of the anterior median fissure (Fig. 3), although it reaches its largest diameter in the cervical and upper thoracic levels and then begins to diminish in size as it descends further down the spinal cord. The posterior spinal arteries run the entire length of the spinal cord and are located in the posterolateral sulci. The anterior and posterior spinal arteries anastomose with each other along the length of the spinal cord via the *arterial vasocorona* (Fig. 4). The vascular supply to the spinal cord is most attenuated between the T3 and T9 levels, and these spinal cord segments are most vulnerable to ischemia.

Like any long tubular structure in the body, the spinal cord receives multiple arterial tributaries that augment the blood supply provided by the three aforementioned spinal arteries. The anterior spinal

artery throughout its length receives anastomotic branches from segmental vessels that enter intervertebral foramina with the ventral and dorsal root fibers. In cervical levels, segmental arteries arise from sources: vertebral arteries, the ascending cervical branch of the inferior thyroid artery, and the deep cervical branch of the costocervical trunk. At thoracic levels, posterior intercostal arteries arising from the descending aorta supply segmental arteries to the spinal cord. At lumbar levels, lumbar arteries originating from the abdominal aorta supply blood, and sacral arteries from internal iliac arteries supply the lowest levels of the spinal cord and cauda equina. Each segmental artery enters an intervertebral foramen to give rise to small posterior and anterior radicular arteries that follow and supply the dorsal and ventral roots (Fig. 4). Periodically, the segmental arteries give rise to *anterior medullary arteries* and *posterior medullary arteries*, which anastomose with the anterior and posterior spinal arteries. Typically, there are three medullary arteries supplying cervical spinal cord levels, two supplying thoracic levels, and two for the lumbar spinal cord.

The *great anterior medullary artery* (of Adamkiewicz), which arises from a posterior intercostal artery located around the 8th to 11th thoracic level, is noticeable because of its large size. This artery is responsible for supplementing the blood supply to the lumbar enlargement. This major contributor to the blood supply of the anterior spinal artery is of clinical importance when the abdominal aorta is clamped for surgery. During surgery, the possibility arises that lumbosacral spinal segments will become infarcted if the anterior spinal artery does not receive enough additional blood from other more caudal segmental arteries to compensate for the temporary loss of blood supply to lumbosacral segments from the great anterior medullary artery. Posterior medullary arteries are smaller and more numerous than are the anterior medullary arteries, with an average of three to five for each spinal cord region. These arteries supplement the blood supply to the dorsal third of the spinal cord.

2.3. Venous Drainage of the Spinal Cord

The veins that drain the spinal cord begin as capillaries within it. These coalesce into *intramedullary veins*, which drain into the more superficial *intradural (pial) veins* located within the pia mater. The venous pattern is variable, but most consistently you will find the following: the *anterior median vein*, located in the anterior median fissure, the *posterior median vein*

located in the posterior median sulcus, *anterolateral veins* located near the exit of ventral roots, and *posterior lateral veins* located in or near the entrance of the dorsal roots. Each of these valveless veins freely communicates with its neighbors forming large anastomotic channels along the surface of the spinal cord. At the base of the skull, the intradural veins unite to form several trunks that drain into the posterior inferior cerebellar veins and vertebral veins of the cranial cavity. The intradural veins also communicate with the *internal vertebral venous plexus* that is contained within the epidural fat found in the epidural space of the vertebral canal along its entire length. The internal vertebral plexus can be divided into an *anterior internal vertebral venous plexus*, located between the vertebral body and the dural sac, and a *posterior internal vertebral venous plexus* between the vertebral arch and the dural sac. The internal vertebral venous plexus drains more superficially into the *external vertebral venous plexus* surrounding the vertebral column. The external vertebral plexus consists of an *anterior external vertebral plexus* around the vertebral bodies and the *posterior external vertebral plexus* lying on the surface of the vertebral arch. Both the anterior and posterior external vertebral plexuses anastomose with each other and drain into the systemic segmental veins, including the deep cervical veins, intercostal veins, lumbar veins, and lateral sacral veins.

The internal vertebral plexus communicates freely with the basilar plexus of the dural venous sinuses, whereas the external vertebral plexus communicates freely with the pelvic plexus of veins. Clinically, these extensive valveless anastomoses allow for passage of infectious material or metastases to travel up and down the length of the vertebral column from the pelvis into the cranium. For example, prostate cancer spreading via this route will lodge and grow within the marrow of the vertebral bodies and can eventually spread to the bones of the cranium.

2.4. Meninges of the Spinal Cord

The spinal cord is covered by three connective tissue layers known as the *meninges* (Fig. 1). The most superficial layer is the *dura mater* ("tough mother"). The spinal dura mater begins at the foramen magnum and extends caudally within the vertebral canal as a sac to the second sacral vertebra. At each intervertebral foramen, the dura mater extends as a *dural sleeve* to cover the dorsal roots, ventral roots, and spinal ganglia. It ends at the external edge of the intervertebral foramen where it becomes

continuous with the epineurium of the spinal nerve. At the caudal end of the vertebral canal, the dura mater ends as the blind-ended *dural sac*. The *arachnoid membrane* (spider-like), the second meningeal layer, is immediately deep to the dura mater and consists of a fine network of connective tissue fibers that extend to the surface of the spinal cord. It follows the contours of the dura mater and is present in the dural sleeves and the dural sac. The *pia mater* ("delicate mother") is the deepest and thinnest meningeal layer and is typically adherent to the surface of the spinal cord, faithfully following its contour. It is a vascular layer containing arteries supplying the spinal cord and veins that drain the spinal cord. The *denticulate ligaments* are irregularly found, sawtooth-like lateral extensions of the pia mater that extend from the side of the spinal cord between the dorsal and ventral roots to anchor to the overlying dura mater/arachnoid membrane (Fig. 3). Tough and fibrous in nature, there are 20 to 22 pairs of denticulate ligaments. Extending caudally from the conus medullaris, in the midst of the *cauda equina*, the pia mater leaves the spinal cord, surrounds a few vestigial neurons, and forms a fine filament, the *filum terminale*. Within the dural sac it is known as the *filum terminale interna*. Once it passes through the dura sac and becomes enveloped in dura mater, it becomes the *filum terminale externa* (also called coccygeal ligament) that exits the sacral hiatus of the vertebral canal and anchors to the dorsum of the coccyx.

Several spaces, either real or potential, are associated with the meninges of the spinal cord. (A potential space is only present due to pathologic conditions or death.) The *epidural space* is superficial to the dura mater and contains significant amounts of fat that protect the spinal cord. It also contains the internal vertebral plexus of veins, which drains blood from the spinal cord to the more superficial external vertebral venous plexus. The *subdural space* is a potential space that lies deep to the dura mater but superficial to the closely adherent arachnoid membrane. In life, cerebrospinal fluid (CSF) pressure keeps the arachnoid pressed against the inner surface of the dural sac, thus closing this space. After death, the loss of CSF pressure causes the arachnoid to collapse upon the surface of the spinal cord revealing the subdural space in the cadaver. The *subarachnoid space* separates the arachnoid membrane from the deeper pia mater. Cerebrospinal fluid, produced by the choroid plexus of the ventricular system, is contained within the subarachnoid space and allows the spinal cord to float within this space.

The epidural and subarachnoid spaces are clinically useful. A *spinal tap* (lumbar puncture) is used to remove CSF from the lumbar cistern of the dural sac. In this procedure, a needle is inserted through skin, epaxial muscles, and the elastic *ligamentum flavum* located between adjacent vertebral laminae. The insertion of the needle is usually between the L3 and L4 laminae. Once through *ligamentum flavum*, the needle passes through the epidural space, dura mater, and the closely adherent arachnoid membrane, and into the subarachnoid space containing CSF. The insertion of the needle into the lumbar cistern below the L3 vertebral body ensures that the adult spinal cord, which ends at the L1-2 intervertebral disk, will not be penetrated and damaged by the needle. The dorsal and ventral spinal roots of the cauda equina are easily pushed aside by the needle and are not damaged. An *epidural block* is performed when anesthesia of the lower body is needed. It is most frequently performed to relieve the pain of childbirth. The anesthetic is delivered into the epidural space that is traversed by the dorsal and ventral roots of spinal nerves. The anesthetic agent rapidly diffuses through the epidural fat and into the thinly myelinated pain fibers thereby temporarily inactivating them.

3. BRAIN

The brain develops from several regions of the rostral neural tube that bulge laterally as neurogenesis proceeds. There is a *forebrain (prosencephalon)*, giving rise to the cerebral cortex, hypothalamus, and thalamus, the *midbrain (mesencephalon)*, which remains as the adult midbrain, and the *hindbrain (rhombencephalon)*, giving rise to the pons, cerebellum, and medulla oblongata. The spinal cord develops from the remainder of the neural tube. The medulla oblongata, pons, and midbrain are collectively described as the *brain stem*.

The brain is located and protected in the cranial cavity. The cranial floor is divided into three horizontal shelves or fossae, from rostral to caudal, which are successively lower (Fig. 5 and Fig. 6). The anterior cranial fossa is composed of the crista galli and cribiform plate of the ethmoid bone, the lesser wing of the sphenoid bone, and the orbital part of the frontal bones. The orbital surface, so named because it forms the roof of the orbit, supports the orbital surface of the frontal cortex. The ethmoid bone on either side of the midline crista galli is perforated (cribiform plate) to allow the olfactory nerves (CN I) to

pass from the olfactory epithelium of the nasal cavity into the paired olfactory bulbs. The olfactory bulbs are directly connected to the temporal cortex by the posteriorly running olfactory tracts.

Posterior to the lesser wing of the sphenoid is the middle cranial fossa. It extends caudally to the petrous ridge of the temporal bone. The temporal cortex's inferior surface rests on the tegmen tympani of the temporal bones, and the temporal poles are tucked into the concavities of the greater wing of the sphenoid bone located beneath the overhanging lesser wing. In the midline, the depression of the sella turcica of the sphenoid bone houses the pituitary and infundibulum of the hypothalamus. Cranial nerves II to VI exit the skull through the superior orbital fissure and several foraminae in the middle cranial fossa (Fig. 6 and Fig. 7).

The posterior cranial fossa is located posterior to the petrous ridge of the temporal bone and is bounded by the mastoid process of the temporal bone laterally with the occipital bone forming the majority of the rest of boundaries. Its floor contains the foramen magnum, which allows the seamless continuation of the brain stem with the spinal cord. The brain stem and the cerebellum are contained in the posterior cranial fossa. Its roof is formed by the *tentorium*

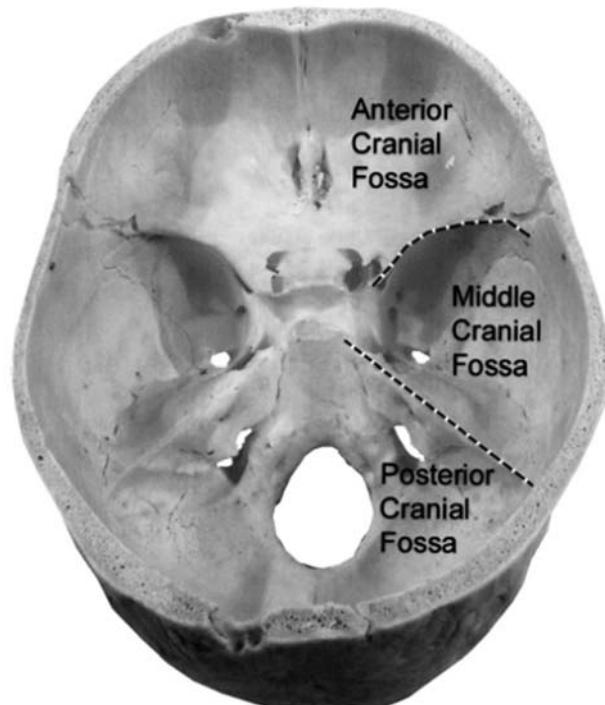


Fig. 5. Floor of the cranial cavity demonstrating the boundaries of the anterior cranial fossa, middle cranial fossa, and posterior cranial fossa.

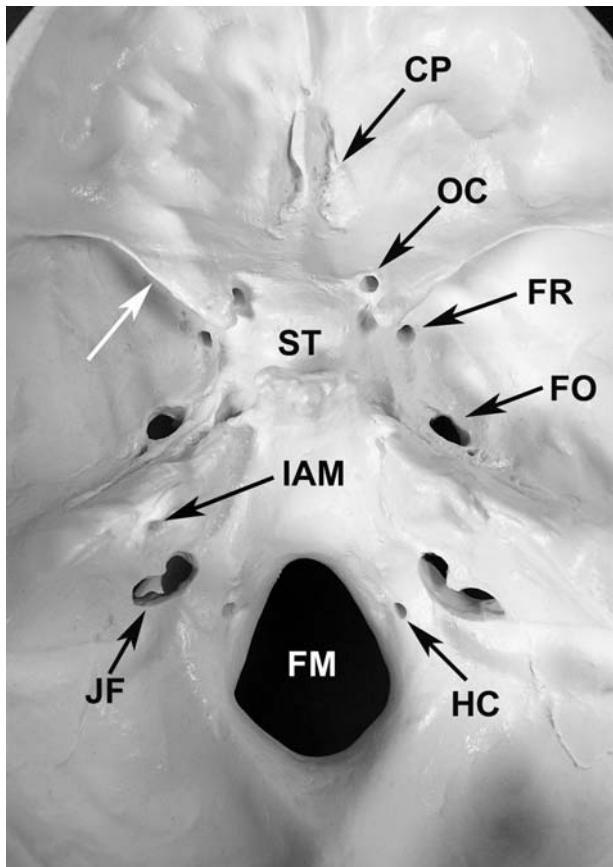


Fig. 6. The interior of the skull base with foraminae labeled that provide entrance or exit of cranial nerves. The olfactory nerves enter the skull through the cribriform plate (CP). The optic nerve enters via the optic canal (OC). The oculomotor, trochlear, ophthalmic division of the trigeminal, and abducens nerves exit the skull through the superior orbital fissure (white arrow) that is hidden by the overhanging lesser wing of the sphenoid bone. The maxillary and mandibular divisions of the trigeminal nerve exit the skull through the foramen rotundum (FR) and the foramen ovale (FO), respectively. The facial and vestibulocochlear nerves exit through the internal acoustic meatus (IAM). The vagus and glossopharyngeal nerves exit the skull through the jugular foramen (JF). The hypoglossal nerve exits via the hypoglossal canal (HC). The accessory nerve enters the skull through the foramen magnum (FM) and then turns and exits through the jugular foramen. The pituitary sits within the sella turcica (ST) of the sphenoid bone.

cerebelli of the dura mater. Cranial nerves VII to XII exit the skull through several foraminae in the posterior cranial fossa (Fig. 6).

3.1. Medulla Oblongata

The medulla oblongata, formed from the myelencephalon and located in the posterior cranial fossa, is

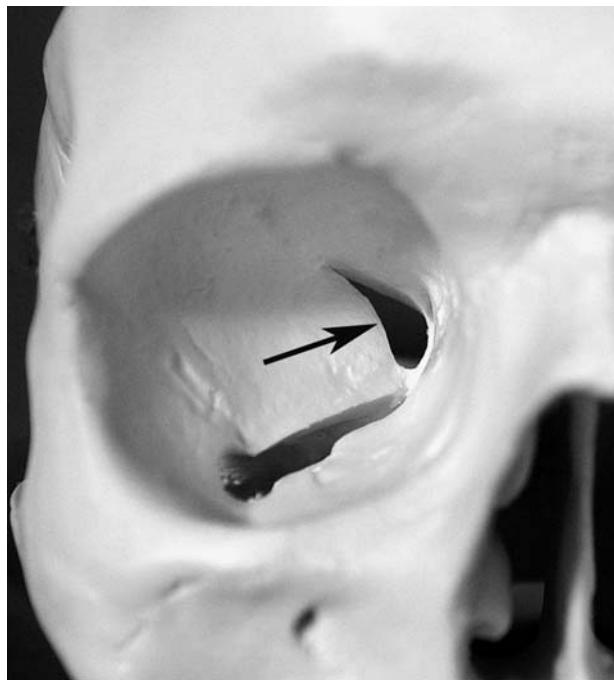


Fig. 7. Anterior view of the skull showing the right orbit. The superior orbital fissure (arrow) provides a conduit for the passage of a number of cranial nerves: oculomotor, trochlear, abducens, and the ophthalmic division of the trigeminal. The inferior orbital fissure is seen in the floor of the orbit, and part of the nasal cavity is visible on the right.

the most caudal portion of the brain stem and is continuous with the spinal cord at the foramen magnum (Fig. 8, Fig. 9, Fig. 10, and Fig. 11). Its anterior surface contains two prominent ridges along its length. The most medial pair of ridges are the *pyramids* formed by the corticospinal tracts, and the more lateral ridges are the *olives*, formed by the inferior olive nuclei. Each pyramid is separated from the other by an *anterior median fissure* and from the more lateral olive by the *anterior lateral sulcus*. The anterior lateral sulcus contains the hypoglossal nerve (CN XII) fibers. The olive is bounded laterally by the *posterior lateral sulcus*, which contains the fibers of the glossopharyngeal nerve (CN IX) and the vagus nerve (CN X). The posterior surface of the medulla contains the tubercle of the nucleus gracilis medially and the tubercle of the nucleus cuneatus laterally (Fig. 9). It opens into a diamond-shaped open region known as the *rhomboid fossa*, which forms the floor of the fourth ventricle. The medulla oblongata is continuous rostrally with the pons. At the pons-medulla junction, the abducens nerve (CN VI) arises medially, and the facial (CN VII) and vestibulocochlear (CN VIII) nerves originate further laterally.

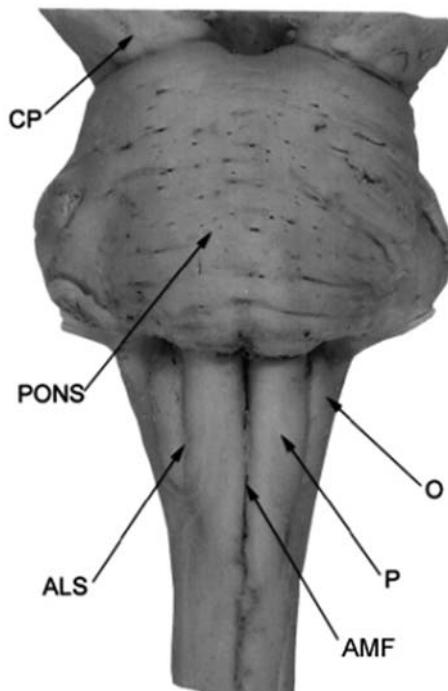


Fig. 8. Anterior surface of the brain stem. The cerebral peduncles (CP) of the midbrain are rostral to the pons. The medulla contains the pyramids (P), separated from the olive (O) by the anterior lateral sulcus (ALS). The pyramids are located on either side of the anterior median fissure (AMF).

The medulla contains the motor nuclei for skeletal muscles innervated by CN IX, X, and XII and autonomic nuclei containing preganglionic PSNS neurons for CN IX and X. The *spinal trigeminal nucleus*, associated with CN V, receives somatic sensory afferents from the head. The spinal trigeminal nucleus extends caudally from the medulla into the first several cervical spinal segments. The medulla also contains the nucleus solitarius that receives visceral sensations from CN IX and X, as well as the special sense of taste from CN V, VII, and X. At the most caudal aspect of the fourth ventricle (i.e., the obex), the area postrema, one of the *circumventricular organs* that lack a blood-brain barrier, senses blood-borne toxins and comprises the chemoreceptive trigger zone for the vomiting reflex. The rostral half of the posterior medulla contributes to the caudal half of the rhomboid fossa (Fig. 9).

3.2. Pons

The pons, formed from the metencephalon and located in the posterior cranial fossa, lies against the basilar portion (clivus) of the occipital bone (Fig. 8, Fig. 9, Fig. 10, and Fig. 11). It is continuous with the

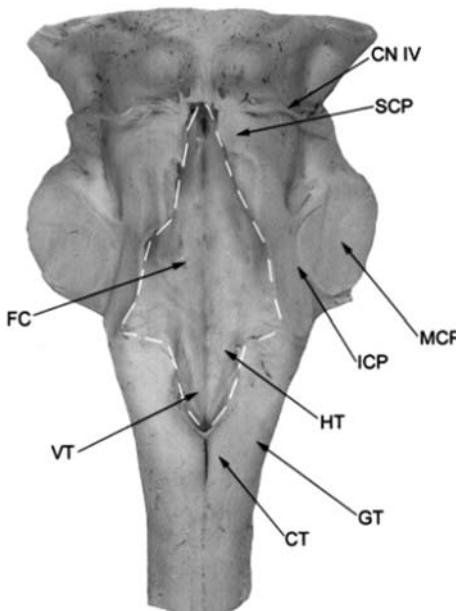


Fig. 9. Posterior surface of the brain stem. The rhomboid fossa (outlined by dashed lines), visible after removal of the cerebellum, forms the floor of the fourth ventricle. The facial colliculus (FC), hypoglossal trigone (HT), and vagal trigone (VT), formed by underlying cranial nerve nuclei, are slight elevations located on the floor of the rhomboid fossa. The cuneate tubercle (CT) and gracile tubercle (GT) are located caudal to the rhomboid fossa and are formed by underlying sensory nuclei. Rostral to the rhomboid fossa, cranial nerve IV (trochlear) is seen originating from the posterior surface of the brain stem. The inferior cerebellar peduncle (ICP), middle cerebellar peduncle (MCP), and superior cerebellar peduncle (SCP), which connect the brain stem to the cerebellum, are visible.

medulla oblongata caudally and the midbrain rostrally. Its anterior region (basis pontis) contains pontine nuclei scattered among major descending tracts (Fig. 8 and Fig. 10). The pons is expanded along its lateral sides for passage of fibers to the cerebellum in the *middle cerebellar peduncles*. The trigeminal nerve (CN V) arises from the anterolateral surface of the middle cerebellar peduncle. The posterior surface of the pons contributes to the floor of the rostral half of the rhomboid fossa of the fourth ventricle (Fig. 9). The posterior pons contains motor nuclei for skeletal muscles innervated by CN V, VI, and VII, an autonomic nucleus containing preganglionic PSNS neurons for CN VII, as well as the *main sensory nucleus* (also known as the *principal nucleus*) associated with CN V.

3.3. Midbrain

The midbrain, formed from the mesencephalon and contained in the posterior cranial fossa, is located

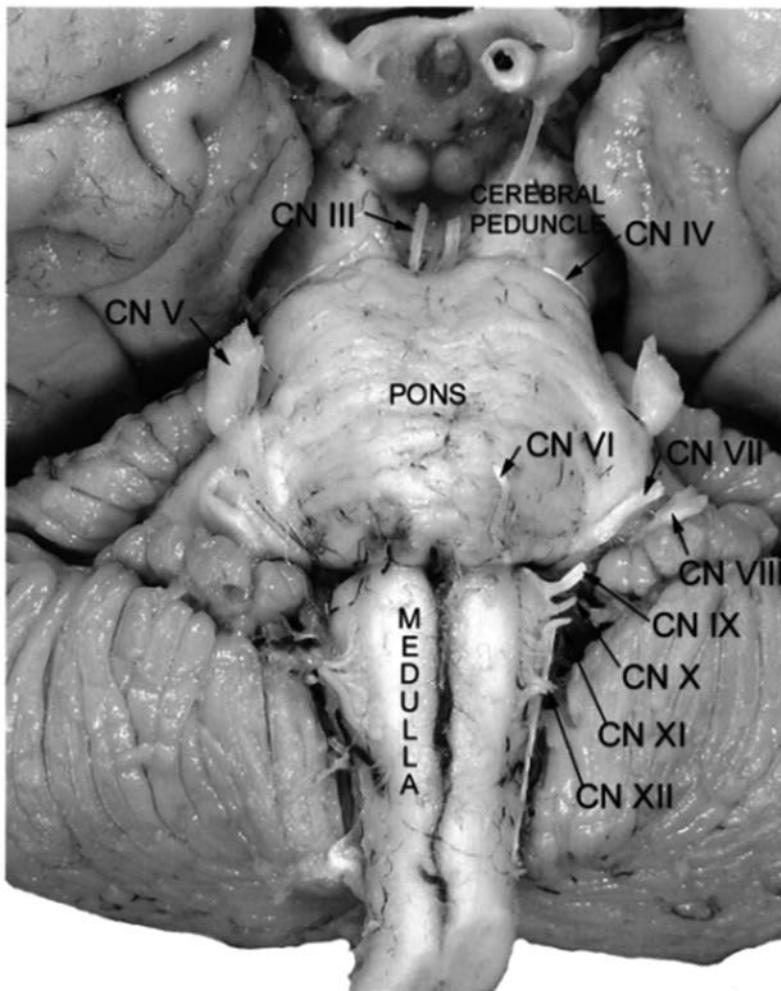


Fig. 10. Anterior surface of the brain stem. The medulla, pons, and cerebral peduncles (anterior representation of the midbrain) are labeled, as well as the individual cranial nerves arising from each region of the brain stem. The midline oculomotor nerve (CN III) arises in the interpeduncular fossa (between the cerebral peduncles) rostral to the pons. The trochlear nerve (CN IV) wraps around the cerebral peduncle from the posterior surface of the midbrain. The trigeminal nerve (CN V) arises from the middle cerebellar peduncle of the pons. The abducens nerve (CN VI) arises from the midline of the pontomedullary junction, and the facial nerve (CN VII) and vestibulocochlear nerve (CN VIII) originate from the lateral region of the pontomedullary junction. The glossopharyngeal nerve (CN IX) and vagus nerve (CN X) arise lateral to the pyramid. The accessory nerve (CN XI) arises from the C1 through C5 spinal cord segments and lies lateral to the Pyramid. The hypoglossal nerve (CN XII) arises in the ventrolateral sulcus between the pyramid and olive.

between the pons caudally and the thalamus and hypothalamus of the diencephalon superiorly (Fig. 10 and Fig. 11). The cerebral aqueduct, the narrowest portion of the ventricular system, passes through it. The anterior region is characterized by the prominent *cerebral peduncles* that comprise part of the corticospinal tract. Between their medial borders there is a CSF-filled depression called the *interpeduncular fossa*. The oculomotor nerves (CN III) arise from the midbrain in the interpeduncular fossa (Fig. 10). The posterior region of the midbrain, also known as the tectum, is composed of the *corpora quadrigemina*, four prominent

tubercles consisting of the rostral *superior colliculi* (colliculus, singular), and the more caudal *inferior colliculi* (Fig. 9 and Fig. 11). The superior and inferior colliculi deal with the special senses of vision and hearing, respectively. The trochlear nerve (CN IV), the only cranial nerve to arise posteriorly from the brain stem, is found just caudal to the inferior colliculus. The midbrain contains motor nuclei for skeletal muscles innervated by CN III and IV, an autonomic nucleus containing preganglionic PSNS neurons for CN III, as well as the somatic sensory *mesencephalic nucleus*, associated with CN V.

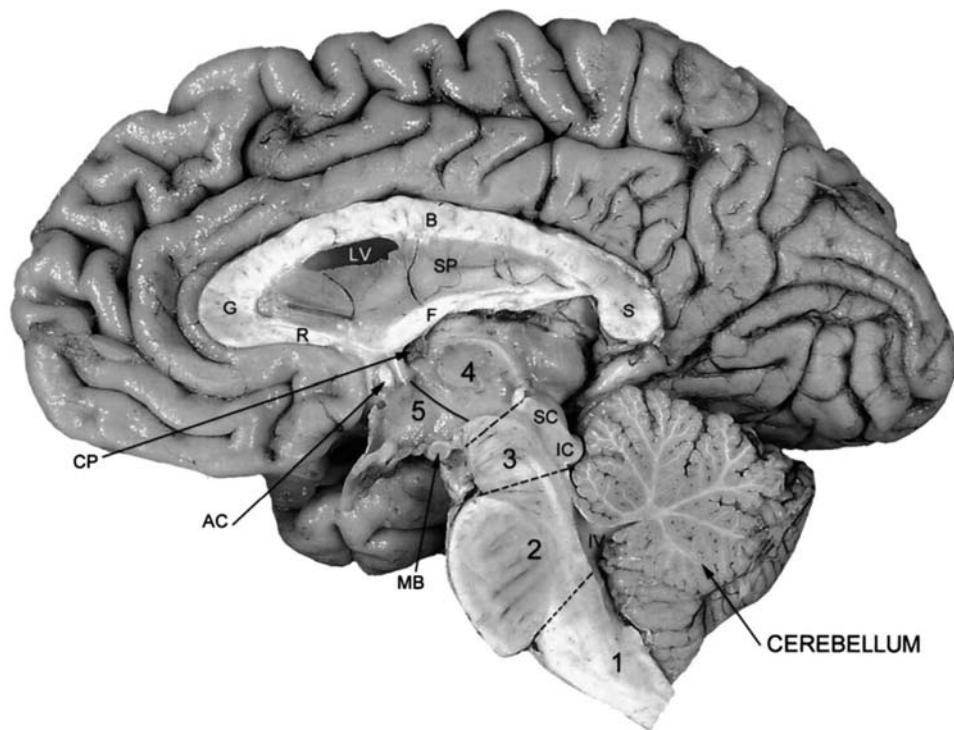


Fig. 11. Midsagittal section of the brain. The brain stem is composed of the medulla oblongata (1), pons (2), and midbrain (3). The cerebellum lies posterior to the caudal brain stem and forms the roof of the fourth ventricle (IV). The corpora quadrigemina, made up of the superior colliculus (SC) and inferior colliculus (IC), is located posterior to the cerebral aqueduct that connects the third ventricle with the fourth ventricle (IV). The diencephalon, containing the thalamus (4) and hypothalamus (5), is rostral to the midbrain and forms the lateral walls of the third ventricle. The mammillary bodies (MB) are hypothalamic nuclei visible on the base of the brain. The anterior commissure (AC) is rostral to the hypothalamus, and the fornix (F) arches over the thalamus. The corpus callosum is composed of the rostrum (R), genu (G), body (B), and splenium (S). The septum pellucidum (SP) separates the lateral ventricle (LV) of each cerebral hemisphere. Choroid plexus (CP), which produces CSF, can be seen protruding through the interventricular foramen that connects the lateral ventricles with the midline third ventricle.

3.4. Cerebellum

The cerebellum (“little brain”) is located in the posterior cranial fossa, immediately deep to the dura mater specialization the *tentorium cerebelli*, and posterior to the pons and medulla (Fig. 11 and Fig. 12). The cerebellum completes the roof of the fourth ventricle along with its extensions, the *superior medullary velum* and the much thinner *inferior medullary velum* (Fig. 12). The best way to visualize the physical arrangement of the cerebellum is to view it as a planar structure folded upon itself as a piece of paper is folded upon itself, so that its inferior edge is brought up in contact with its superior edge. The cerebellum is notable for its extensive, transversely oriented *folia* (Fig. 11 and Fig. 12). It is composed of two lateral *cerebellar hemispheres* that are continuous with the midline *vermis*. The separation of vermis from cerebellar hemispheres is less obvious on the superior surface than on the inferior surface of the cerebellum.

Several fissures separate the cerebellum into its major lobes. The two most important fissures—the primary fissure and the posterolateral fissures—divide the cerebellum into three major lobes: anterior, posterior, and flocculonodular (Fig. 12). The *primary fissure* separates the anterior lobe from the posterior lobe, and the *posterolateral fissure* separates the posterior lobe from the flocculonodular lobe. The numerous remaining fissures help to separate the cerebellum into additional *lobules*. The cerebellar hemisphere’s lobules from its rostral to caudal edges are the anterior quadrangular, posterior quadrangular, superior semilunar, inferior semilunar, gracile, biventer, tonsil, and flocculus. The vermis lobules from its rostral to caudal edges are the lingula, central, culmen, declive, folium, tuber, pyramis, uvula, and nodulus. Additional details concerning cerebellar anatomy and cytoarchitecture are contained in a separate chapter of this volume.

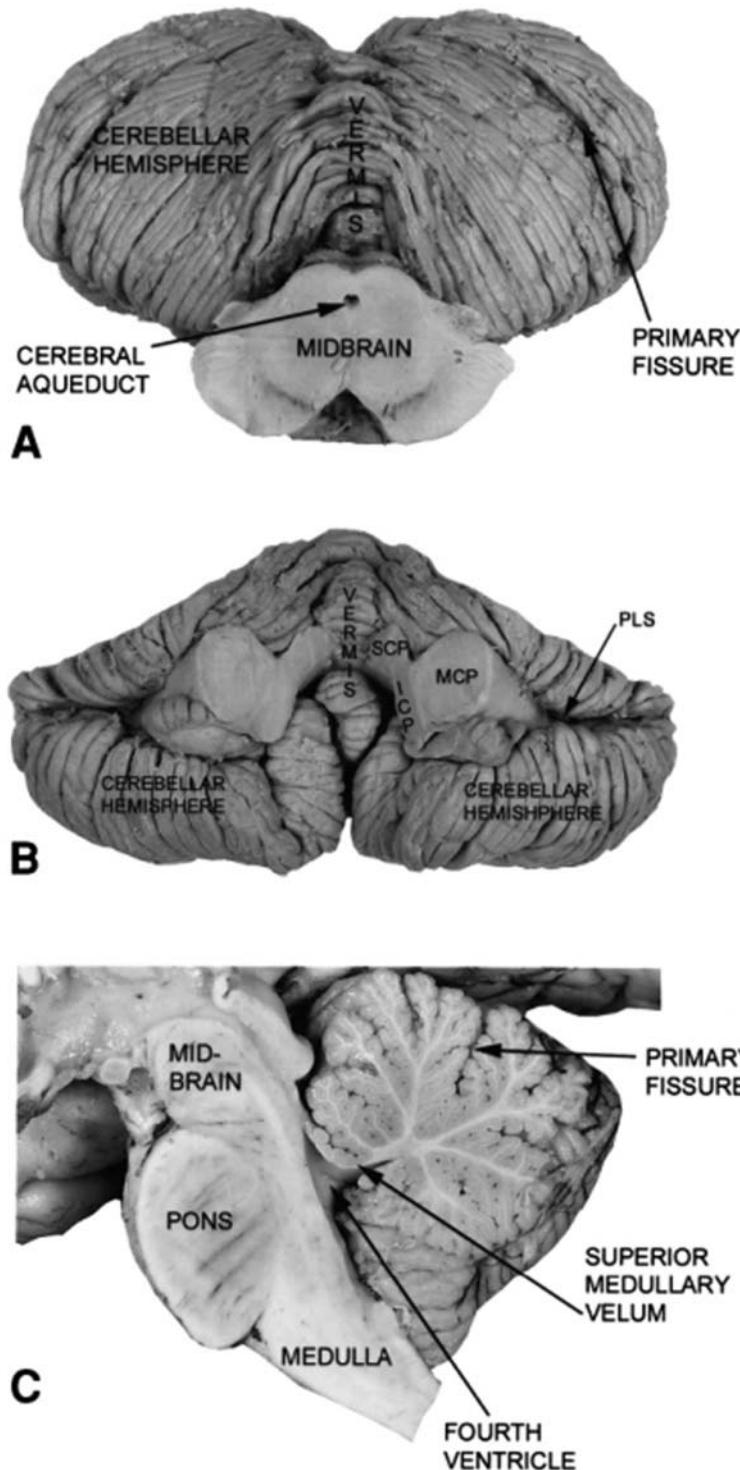


Fig. 12. (A) Anterior (superior) view of the cerebellum. The cerebellum, with its cerebellar hemispheres and midline vermis, lies posterior to the midbrain. The primary fissure separates the anterior lobe from the posterior lobe. The cerebral aqueduct is located in the midbrain. (B) Inferior view of the cerebellum. The two cerebellar hemispheres are present laterally, and the vermis is located in the midline. The posterolateral sulcus (PLS) separates the posterior lobe from the flocculonodular lobe. The inferior cerebellar peduncle (ICP), middle cerebellar peduncle (MCP), and superior cerebellar peduncle (SCP) are visible. (C) Midsagittal view of the cerebellum and brain stem. The cerebellum lies posterior to the pons and medulla and defines the roof of the fourth ventricle by its superior medullary velum and much thinner inferior medullary velum. The primary fissure separates the anterior lobe from the posterior lobe. The gross appearance of the sagittally sectioned cerebellum is termed the “arbor vitae” (tree of life) because of its resemblance to a tree.

Cerebellar development occurred in three distinct phylogenetic phases. The archicerebellum was the first and most primitive portion of the cerebellum to develop. It consists of the flocculonodular lobe whose primary function is the subconscious control of equilibrium. The paleocerebellum developed next and consists of the anterior cerebellar lobes and the intervening portion of the vermis. This portion of the cerebellum receives massive spinal inputs and is involved with the coordination of limb movements. The most recent part of the cerebellum to develop, the neocerebellum, is composed of the posterior cerebellar lobes and the intervening portion of the vermis. The neocerebellum has very strong connections with the cerebral cortex and is involved with learning and storing the sequential components of skilled movements (e.g., keyboarding or playing an instrument).

3.5. Forebrain

The diencephalon and telencephalon comprise the forebrain (prosencephalon). The diencephalon is composed of the thalamus, hypothalamus, and *epithalamus* and is located between the midbrain caudally and the cerebral cortex rostrally (Fig. 11 and Fig. 13). (Although not obvious in the mature brain, if one considers CNS development, the embryonic telencephalon is situated rostral to the

diencephalon in the neural tube.) The thalamus is located posterior to the hypothalamus, and the epithalamus is posterior to the thalamus (Fig. 11 and Fig. 13). Both the thalamus and hypothalamus are subdivided into numerous nuclei, each of which serve a particular function, and are discussed in detail in separate chapters. Posterior to the thalamus, the midline, glandular pineal body and the paired habenular nuclei comprise the epithalamus. The third ventricle separates the two sides of the diencephalon; but frequently during development, the two thalami touch each other and fuse together to form the *massa intermedia*. There is no specialized function associated with this thalamic tissue bridging the third ventricle. On the lateral wall of the third ventricle, the hypothalamic sulcus (the adult representation of the embryonic sulcus limitans) separates the hypothalamus from the thalamus (Fig. 13). The inferior aspect of the hypothalamus connects to the pituitary through its tuberal (infundibular) region, which is located immediately posterior to the optic chiasm. The posterior pituitary (neurohypophysis) is a developmental extension of the diencephalon, whereas the anterior pituitary (adenohypophysis) arises from Rathke's pouch in the roof of the mouth and secondarily becomes attached to the posterior pituitary. Posterior to the tuberal region a pair of small swellings, known as

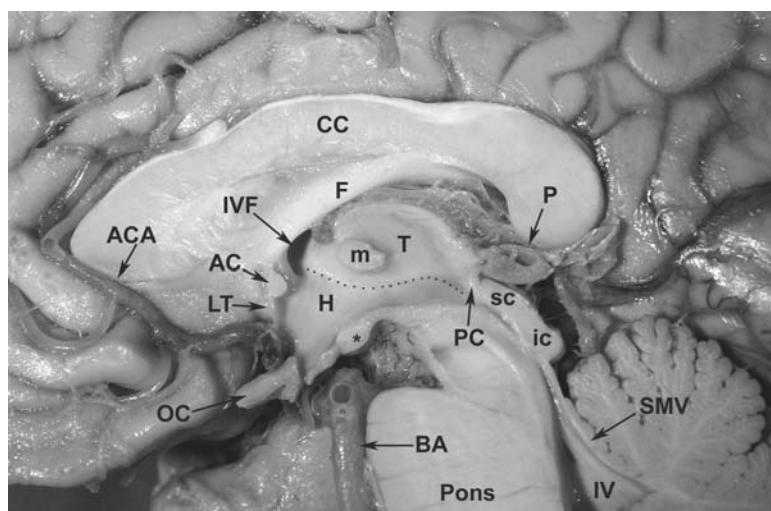


Fig. 13. A midsagittal section of the brain showing details of the diencephalic region. The corpus callosum (cc) lies superior to the diencephalon, which consists of the hypothalamus (H), massa intermedia (m), thalamus (T), the pineal gland (P), and mammillary bodies (*asterisk*). The hypothalamic sulcus is marked by the dotted line. Also visible are the anterior commissure (AC), posterior commissure (PC), lamina terminalis (LT), optic chiasm (OC), anterior cerebral artery (ACA), and fornix (F). The interventricular foramen (IVF), which connects a lateral ventricle with the midline third ventricle, is visible. In the midbrain, the superior colliculus (sc) and inferior colliculus (ic) are seen immediately posterior to the cerebral aqueduct (not labeled), which connects the third ventricle with the fourth ventricle (IV). The superior medullary vellum (SMV) of the cerebellum, which forms part of the roof of the fourth ventricle, is seen, as is the basilar artery (B) on the anterior surface of the pons.

mammillary bodies, are formed by the mammillary nuclei of the hypothalamus (Fig. 11 and Fig. 13). Finally, the retina, optic nerves, and optic tracts are extensions of the diencephalon.

The two cerebral cortices (telencephalon) comprise the majority of the brain. Each cerebral cortex is characterized by prominent raised areas called *gyri* (gyrus, singular) that are separated from each other by *sulci* (sulcus, singular), which are grooves that invaginate the cortex (Fig. 14, Fig. 15, Fig. 16, Fig. 17, and Fig. 18). The cerebral cortex is composed of an outer layer of gray matter, where the majority of neuronal cell bodies are located, and a deeper layer of white matter formed by myelinated and unmyelinated axons arising from the neuronal cell bodies. The two cerebral cortices are separated from one another on their superior side by the *longitudinal fissure* containing the dura mater specialization, the *falx cerebri*. Deep to the falx cerebri, each cerebral hemisphere is connected to the other by the massive *corpus callosum* and a much smaller *anterior commissure*, both of which are composed of myelinated axons. Both of these structures have their embryonic origin from the *lamina terminalis* to which they remain attached in adulthood. The lamina terminalis forms the anterior boundary of the hypothalamus and is the adult representation of the rostral end of the neural tube (Fig. 13).

The cerebral cortex is divided by sulci or fissures into five lobes: the frontal, parietal, temporal, occipital, and insular cortices (Fig. 14, Fig. 15, Fig. 16,

Fig. 17, and Fig. 18). Several major sulci separate the major lobes from one another. The frontal lobe is separated from the parietal lobe by the *central sulcus*, a vertically running sulcus that begins at the longitudinal fissure in the midline and ends just short of the *lateral sulcus* on the lateral surface of the brain. The lateral sulcus separates the temporal lobe from the frontal cortex and parietal cortex. The parietal cortex on the lateral surface of the cerebrum is bounded by the central sulcus rostrally and is poorly delineated from the occipital lobe by drawing an imaginary line beginning at the shallow indentation of the *parieto-occipital notch* and coursing superiorly to the *parieto-occipital sulcus*. On the medial surface of the brain, the parieto-occipital sulcus clearly defines the boundary between the occipital and parietal lobes (Fig. 15 and Fig. 18). The insular cortex lies deep inside the lateral sulcus and is most easily seen when the overlapping portions of the temporal, frontal, and parietal cortices are gently pulled aside (Fig. 16).

The *frontal cortex* is the most rostral portion of the cerebral cortices and extends posteriorly to the central sulcus. It contains four prominent gyri on its lateral surface separated by corresponding sulci (Fig. 17). Three of its gyri (the superior, middle, and inferior frontal gyri) are horizontally oriented at the rostral end of the frontal cortex. The fourth gyrus (precentral gyrus) is vertically oriented caudal to the first three gyri and is the site of the *primary motor cortex*. The superior frontal gyrus begins anterior to

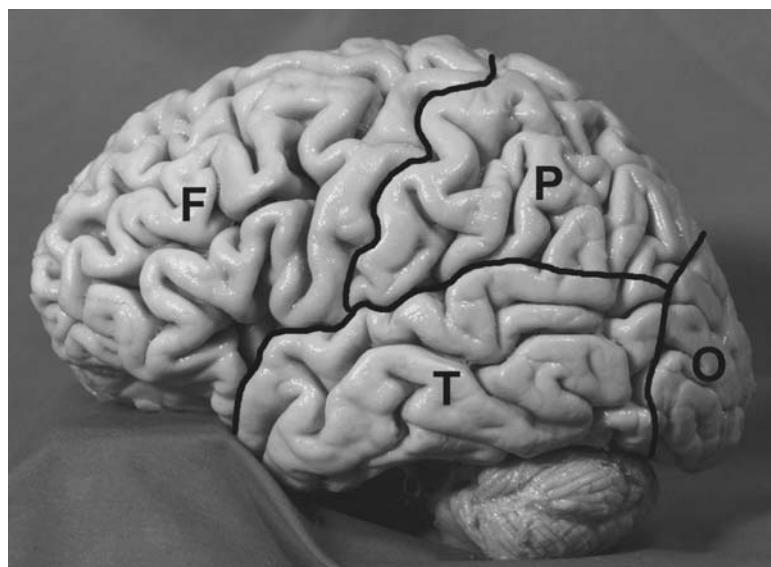


Fig. 14. A lateral view of the cerebral cortex showing the location of the frontal (F), parietal (P), temporal (T), and occipital (O) lobes. The cerebellum is visible beneath the temporal lobe.

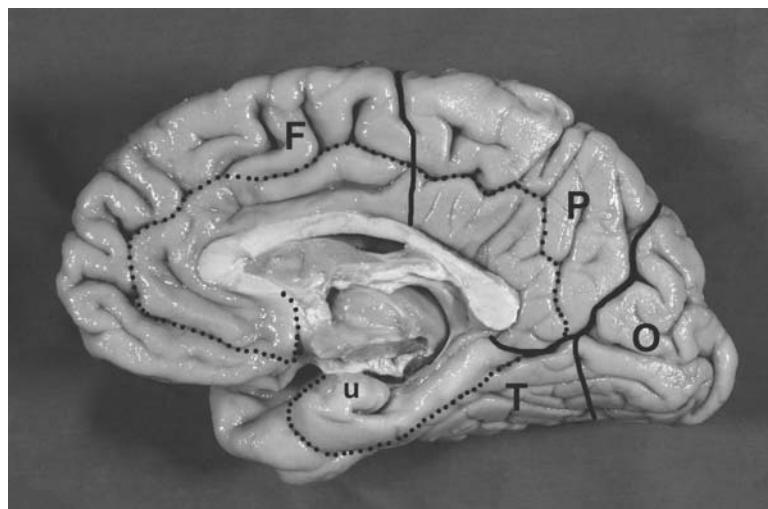


Fig. 15. A midsagittal view of the brain with the brain stem and cerebellum removed. The frontal (F), parietal (P), temporal (T), and occipital (O) lobes can be seen. The cortex contained with the *dotted line* is considered by some to be a separate lobe called the “limbic lobe,” which composes part of the limbic system. The uncus (u) of the temporal lobe is labeled.

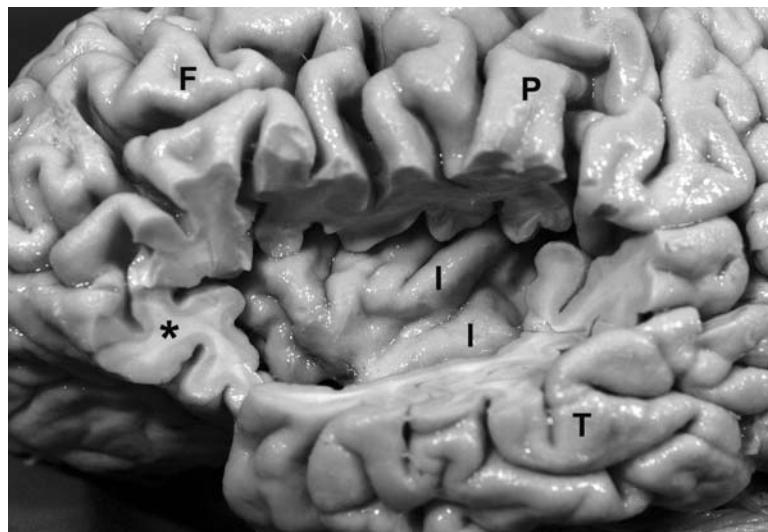


Fig. 16. Insular cortex. The opercula of the frontal (F), parietal (P), and temporal (T) cortices have been dissected away to reveal the underlying insula. The insula contains gyrus longus insulae (I) and several gyri brevi insulae (not labeled). The dissection shows that cortical white matter (*asterisk*) lies deep to the gray matter.

the precentral gyrus and ends at the rostral pole of the brain. It is separated from the wide middle frontal gyrus by the superior frontal sulcus. The middle frontal gyrus that is immediately inferior to the superior frontal gyrus is the broadest of the frontal gyri and also begins at the anterior border of the precentral gyrus. The inferior frontal sulcus is its inferior border, separating it from the inferior frontal gyrus. The inferior frontal gyrus is continuous inferiorly with the lateral orbital gyrus that is in contact with the orbital floor of the anterior cranial fossa. The frontal cortex

on its inferior (orbital) surface is many times composed of four gyri that form an “H.” These four gyri are the lateral, anterior, posterior, and medial orbital gyri. The gyrus rectus is most medial, lying immediately lateral to the longitudinal fissure. The olfactory bulb and olfactory tract lie in the olfactory groove located between the gyrus rectus and the more lateral orbital gyri.

The *temporal cortex* on its lateral surface lies inferior to the lateral sulcus, separating it from the frontal and parietal cortices (Fig. 14 and Fig. 17). Posteriorly,

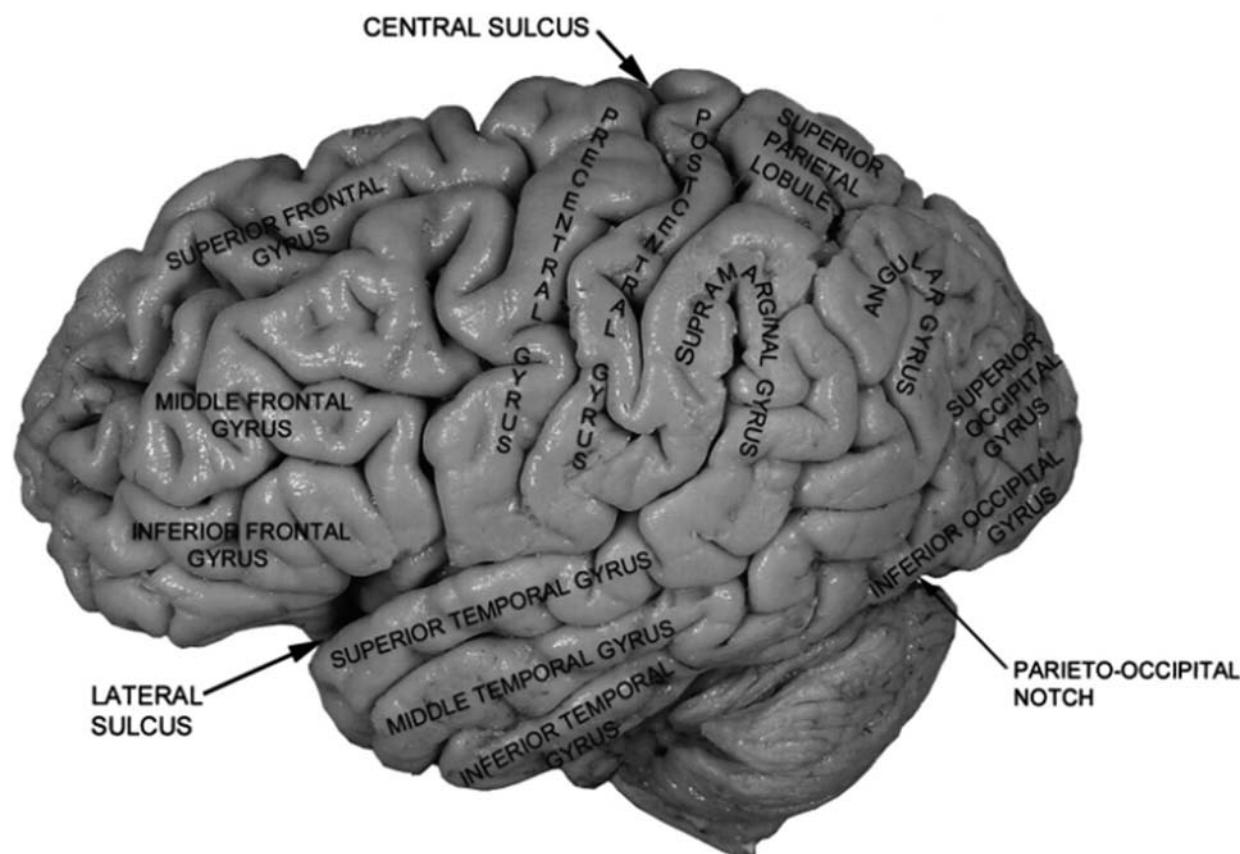


Fig. 17. Cerebral cortex anatomy. The lateral surface of the cerebrum demonstrating the major gyri. The sulci and notches that separate the lobes are labeled.

it is continuous with the parietal cortex. The temporal cortex contains three horizontally oriented gyri: the superior, middle, and inferior temporal gyri. Located on the superior surface of the superior temporal gyrus, and hidden in the lateral sulcus, is the transverse gyrus. The transverse gyrus is the site of the *primary auditory cortex*. The superior temporal sulcus separates the superior temporal gyrus from the middle temporal gyrus, and the inferior temporal sulcus separates the middle temporal gyrus from the inferior temporal gyrus. The temporal cortex continues inferiorly and medially along the base of the brain as a lateral occipitotemporal gyrus, a medial occipitotemporal gyrus, and the most medial parahippocampal gyrus. On the anteromedial surface of the parahippocampal gyrus there is a bulge, the *uncus*, formed by the underlying nuclei of the amygdala (Fig. 15 and Fig. 18).

The *parietal cortex* is located between the frontal cortex anteriorly and the occipital cortex posteriorly (Fig. 14 and Fig. 17). It is separated from the frontal cortex by the central sulcus and from the occipital

cortex by a vertical line drawn from the parieto-occipital notch to the parieto-occipital sulcus. The most prominent gyrus is the vertically oriented postcentral gyrus immediately posterior to the central sulcus. The postcentral gyrus is the site of the *primary sensory cortex*. The remainder of the parietal cortex lies posterior to the postcentral gyrus and is divided into a superior parietal lobule and an inferior parietal lobule. They are separated from each other by the intraparietal sulcus. On the lateral surface, the inferior parietal lobule can be divided into the supramarginal gyrus that arches over the posterior end of the lateral fissure and the angular gyrus that arches over the superior temporal sulcus (Fig. 17).

On the lateral surface of the brain, the *occipital cortex* appears as a continuation of the parietal cortex (Fig. 14 and Fig. 17). Only on the inferior edge of the cortex can a separation be discerned, where a slight indentation, the parieto-occipital notch (or preoccipital notch) is present. The remainder of the lateral surface of the occipital cortex has poorly defined gyri, although a superior occipital gyrus and an inferior

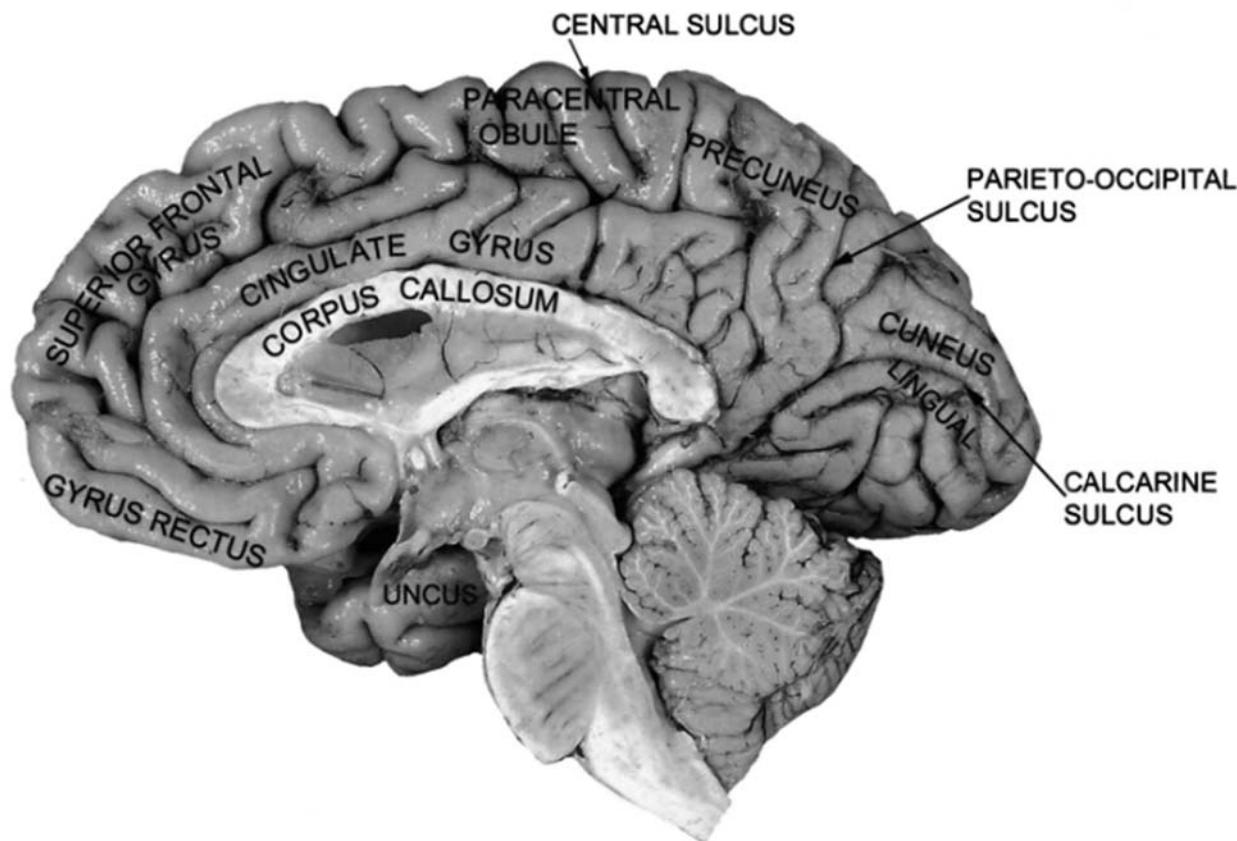


Fig. 18. A midsagittal section of the brain with the gyri and major sulci labeled.

occipital gyrus are often described. On its medial surface, the vertically oriented parieto-occipital sulcus clearly separates the occipital lobe from the more anterior parietal cortex (Fig. 15 and Fig. 18). The horizontal calcarine sulcus separates several small gyri superior to it, collectively known as the cuneus, from the more inferior lingual gyrus. The occipital cortex that lies immediately superior or inferior to the calcarine sulcus is the site of the *primary visual cortex*.

The *insular cortex* is located deep within the lateral sulcus. It is composed of two or more gyri brevi insulae and two or more gyri longus insulae (Fig. 16). The middle cerebral artery passes into the lateral sulcus across the surface of the insula. The portions of the frontal, temporal, and parietal cortices that form the border of the lateral sulcus, and therefore cover the insula, are described as the operculum. The functions of the insular cortex have not been as well investigated as other cortical areas, but studies suggest it deals with nociception and autonomic functions.

Some investigators consider the cerebral cortex has an additional lobe called the *limbic lobe* (Fig. 15). It is composed, in part, of portions of the medial aspects

of the frontal, parietal, and temporal lobes. The limbic lobe is a C-shaped structure surrounding the corpus callosum whose major cortical components include the cingulate gyrus and the parahippocampal gyrus. The limbic lobe forms part of the *limbic system*, which is an evolutionarily primitive part of the brain involved with the primal drives of hunger, reproduction, enemy avoidance, and memory formation.

3.6. Arterial Supply of the Brain

The arterial supply to the CNS is unusual in that the arteries branch over the surface before entering into the parenchyma, whereas in other viscera arteries enter the organ, usually at a singular location, and then branch internally. Like the vascular supply to other parts of the body, variation exists in the vascular territories supplied by an artery. Therefore, all texts will differ slightly when describing CNS vascular distributions. The brain receives its blood supply from two paired arterial sources, the vertebral arteries and the internal carotid arteries (Fig. 19). The *vertebral arteries*, arising from the subclavian arteries at the base of the neck, ascend through the

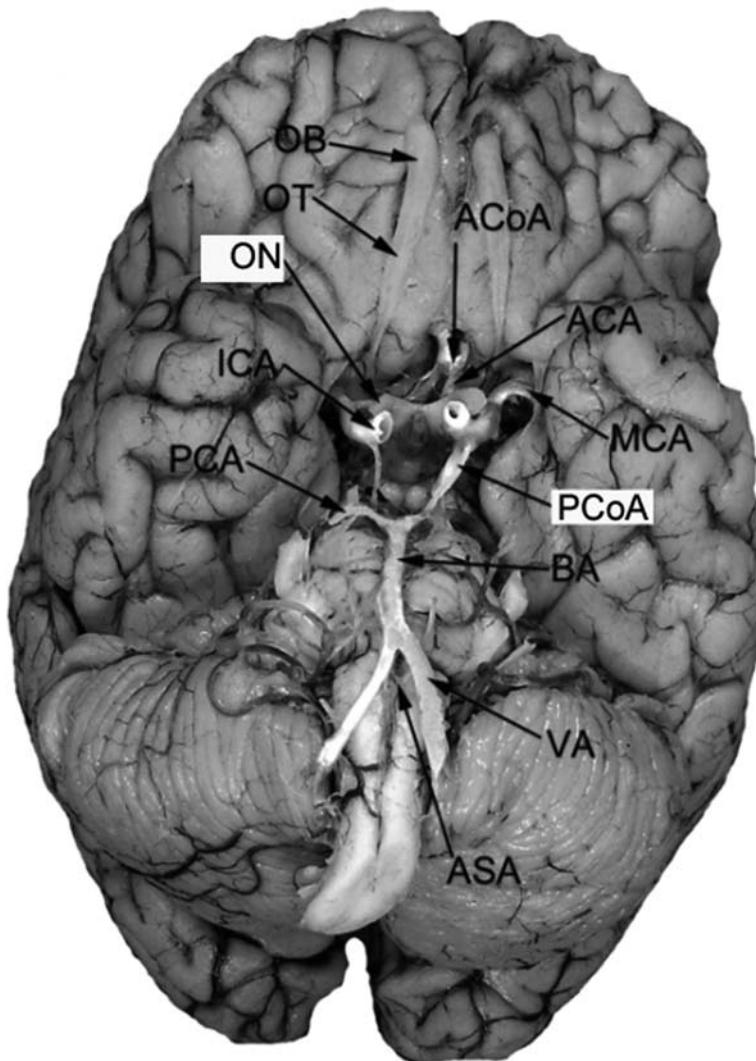


Fig. 19. Inferior surface of the brain, demonstrating anastomotic connections forming the circle of Willis and its associated arterial supply. The two vertebral arteries (VA) unite to form the basilar artery (BA). The anterior spinal artery (ASA) arises from the individual vertebral arteries prior to their union and courses caudally the length of the spinal cord. The basilar artery terminates as the posterior cerebral arteries (PCA). The PCA communicates with the internal carotid artery (ICA) via the posterior communicating artery (PCoA). The middle cerebral artery (MCA) originates from the internal carotid artery and enters the lateral sulcus, and the anterior cerebral artery (ACA) also arises from the internal carotid artery to enter the longitudinal fissure. A short vessel, the anterior communicating artery (ACoA), connects the two anterior cerebral arteries in the longitudinal fissure. In addition to the vascular supply, the olfactory bulb (OB), olfactory tract (OT), and optic nerves (ON) are identified.

transverse foraminae of the upper six cervical vertebrae to enter the foramen magnum at the base of the skull. Once inside the cranial cavity, the two vertebral arteries unite on the anterior surface of the brain stem to form a single *basilar artery*. Prior to their union, they each give rise to an arterial branch that joins with its opposite member to form the *anterior spinal artery*, which descends back through the foramen magnum to run along the anterior surface of the spinal cord.

Each vertebral artery gives rise to a *posterior inferior cerebellar artery* that supplies the posterior surface of the cerebellum. The vertebral, anterior spinal, and posterior inferior cerebellar arteries also supply vascular territories within the medulla.

The singular *basilar artery* begins at the pontomedullary junction and courses along the anterior surface of the pons and midbrain until it terminates as the posterior cerebral arteries just rostral to the origin

of the oculomotor nerves (Fig. 19). Before its termination, the basilar artery gives off several paired branches. Its first branch is typically the *anterior inferior cerebellar artery* that supplies the medial and lateral surface of the cerebellum as well as a portion of the pons. Multiple *pontine branches* also supply the pons as well as the caudal aspect of the midbrain. The *superior cerebellar artery* arises from the basilar artery prior to the oculomotor nerve's origin and is responsible for supplying the superior surface of the cerebellum and part of the midbrain.

The *posterior cerebral arteries*, which course around the cerebral peduncles to reach the posterior surface of the brain stem and inferior surface of the cerebrum, are the final pair of branches from the basilar artery. The posterior cerebral arteries connect with the internal carotid arteries via *posterior communicating arteries*. The posterior cerebral arteries supply blood to the occipital cortex and the ventral surface of the temporal lobe (Fig. 20B, D).

The *internal carotid artery*, one of two terminal branches of the common carotid artery, begins at

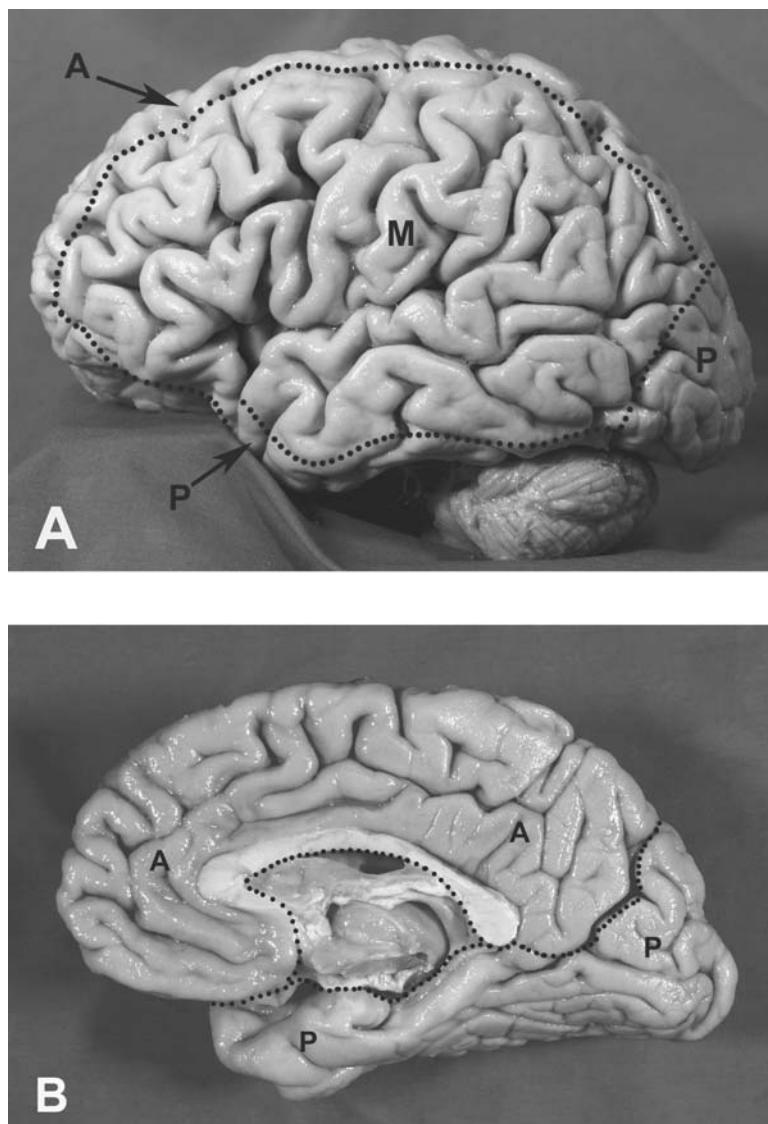


Fig. 20. Views of the cerebral cortex showing the vascular territories supplied by the Anterior (A), Middle (M), and Posterior (P) cerebral arteries. **(A)** Lateral view. **(B)** Midsagittal view with brain stem and cerebellum removed. **(C)** Superior view. Note that the cortical region supplied by the anterior cerebral artery extends over the vertex of the frontal and parietal cortices. **(D)** Inferior view with the pons, medulla, and cerebellum removed. The midbrain has been sectioned. In all views, note that the posterior cerebral artery is the singular source of blood to the occipital cortex.

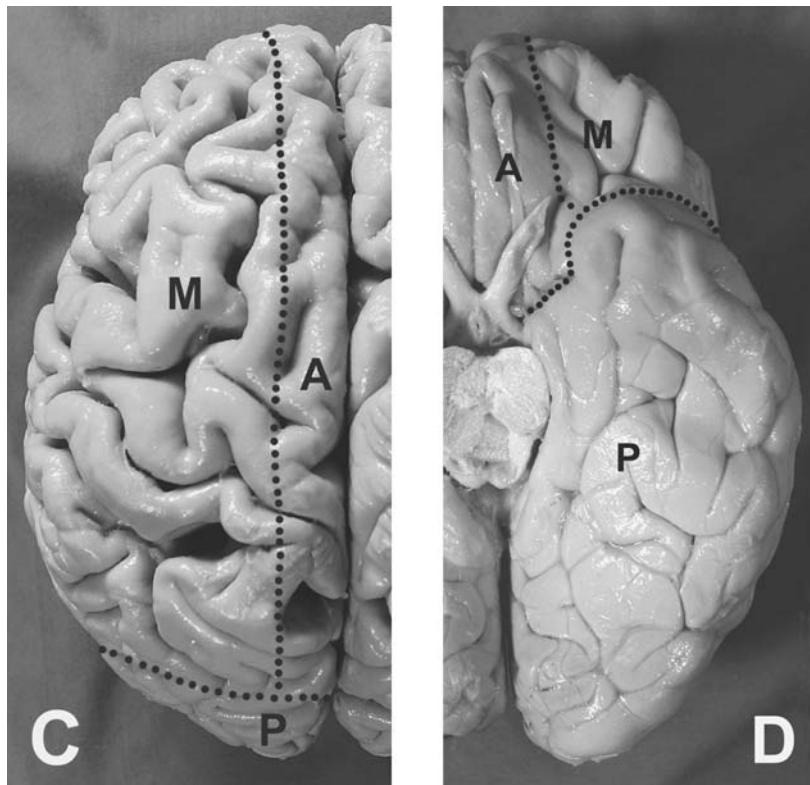


Fig. 20. (Continued).

the C4 vertebral level of the neck. It ascends through the neck without forming any branches and enters the skull through the serpentine-shaped carotid canal of the temporal bone to enter the cranial cavity. In the cranial cavity, it is first located in the cavernous sinus of the sella turcica. Its first branch is the *ophthalmic artery*, which supplies the contents of the orbit. The internal carotid artery passes through the dural extension called the *diaphragma sellae* and immediately terminates as three branches to supply the cerebrum and diencephalon. The posterior communicating arteries course posteriorly to anastomose with the posterior cerebral arteries (Fig. 19).

The *middle cerebral artery* arises from the lateral side of the internal carotid artery and courses laterally and posteriorly to enter the lateral fissure. Its major branches can be divided into striate branches and cortical branches. The lenticulostriate arteries are numerous and are responsible for blood supply to the striatum. Cortical branches (Fig. 20A–D) supply much of the lateral surface of the cerebral cortex. The distribution includes the lateral orbital surface, and the following gyri: precentral, middle frontal, inferior frontal, superior parietal, inferior, parietal, supramarginal, angular, superior temporal, and middle temporal.

The *anterior cerebral artery* follows the corpus callosum—first anteriorly and superiorly and then posteriorly in the longitudinal fissure to supply medial surfaces of the cerebral cortex with the exception of the occipital lobe (Fig. 13 and Fig. 20A–D). In the first part of longitudinal fissure along the anterior surface, the two anterior cerebral arteries are connected by a very short *anterior communicating artery*. Central branches of the anterior cerebral artery supply the septum pellucidum, corpus callosum, and rostral portions of the basal ganglia. Cortical branches supply the olfactory bulb, medial surfaces of the frontal and parietal cortex (gyrus rectus, medial orbital gyrus, superior frontal gyrus, cingulate gyrus, and the precuneate gyrus). The cortical branches of the anterior cerebral artery extend over the vertex of the cerebrum and supply blood to the superolateral aspects of the frontal and parietal lobes. It is clinically important to note that where the vascular regions of the anterior, middle, and posterior cerebral arteries overlap, there are cortical regions called *border zones*. The border zone regions are at increased risk during a hypoxic episode as this is where the smallest terminal arterial branches reside and the chances for an infarction the greatest.

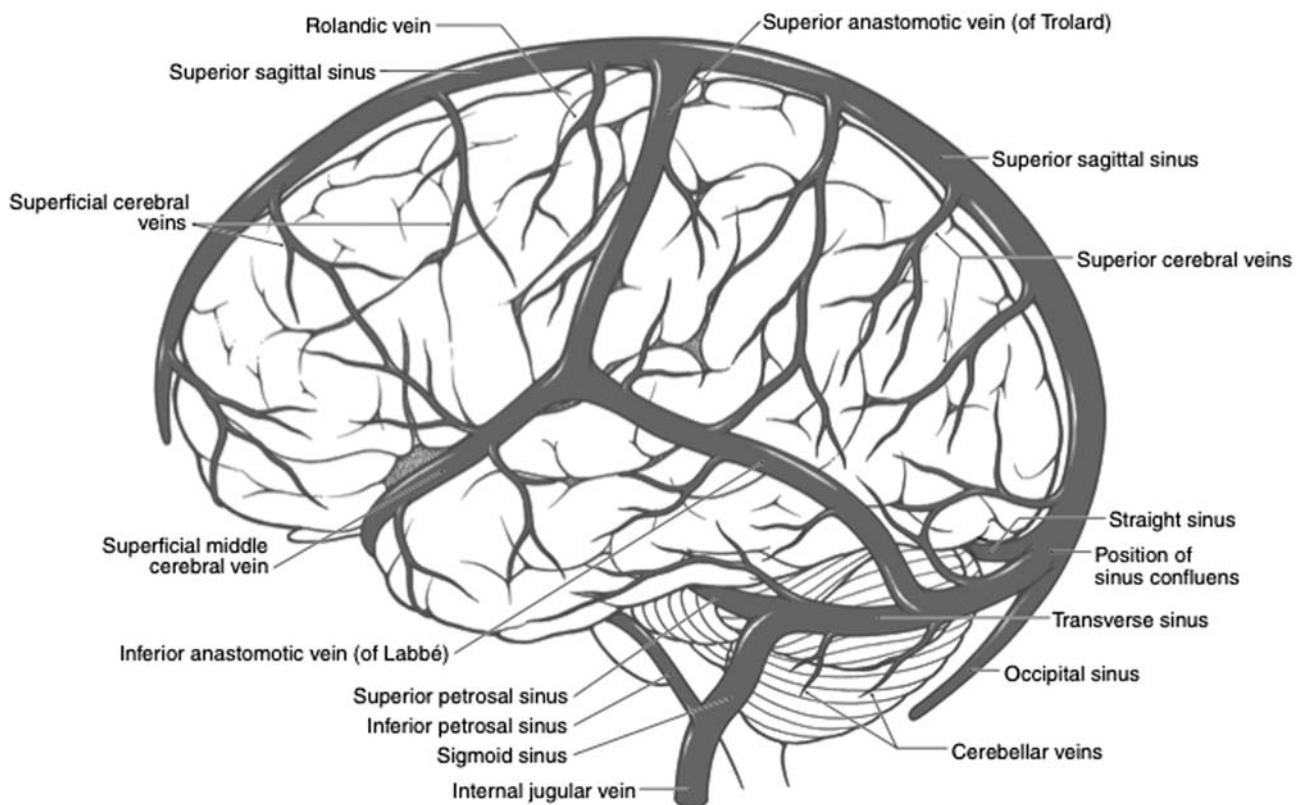


Fig. 21. Veins and dural venous sinuses of the brain. Blood flows from the superficial veins draining the cerebral cortex into dural venous sinuses. Ultimately, the dural venous sinuses drain into the internal jugular veins that originate at the jugular foramen. (Used with permission, from *Neuroanatomy An Atlas of Structures, Sections, and Systems*, 7th ed., by Duane Haines New York: Lippincott Williams and Wilkins.)

An anastomotic channel known as the *circle of Willis* is formed from branches of the internal carotid and vertebral artery systems (Fig. 19). The arteries that contribute to the circle of Willis are the internal carotid arteries, anterior cerebral arteries, anterior communicating artery, posterior communicating arteries, and the posterior cerebral arteries. Frequently, the diameters of the paired arteries vary on either side of the brain so it is not unusual for the circle of Willis to be asymmetrical.

3.7. Venous Drainage of the Brain

Veins that drain the brain are tributaries of dural sinuses that ultimately empty into the internal jugular vein that originates at the jugular foramen (Fig. 6 and Fig. 21). They begin deep within the parenchyma of the brain and drain superficially and can be divided into cerebral veins, cerebellar veins, and veins of the brain stem. The cerebral veins are composed of internal cerebral veins that drain the deep regions of the cerebrum and external cerebral veins located in the sulci and gyri. The superficial cerebral veins, located within the pia mater, are further divided into *superior cerebral veins*,

middle cerebral veins, and *inferior cerebral veins*. The superior cerebral veins are located on the superolateral and medial surfaces of the cerebral cortex within their sulci and drain directly into the superior sagittal sinus. These veins have to span or “bridge” the subarachnoid space before they empty into the superior sagittal sinus (Fig. 22). This represents a site of weakness, and blows to the head (e.g., from a motor vehicle accident or a fall) can tear a *bridging vein* away from the superior sagittal sinus. This results in low-pressure bleeding into the subdural space located between the dura mater and the arachnoid. (Like in the dural sac, the subdural space is a potential space that is only formed after death or if bleeding pushes the underlying arachnoid away from the dura mater.) In elderly individuals, age-related atrophy of the brain can secondarily enlarge the subarachnoid space, stretching the bridging veins and putting them at greater risk to tearing with head trauma.

The *middle cerebral vein* drains the lateral surface of the cerebral cortex, where they empty into the superior sagittal sinus via the *superior anastomotic vein* (Trolard’s vein) or into the transverse sinus by

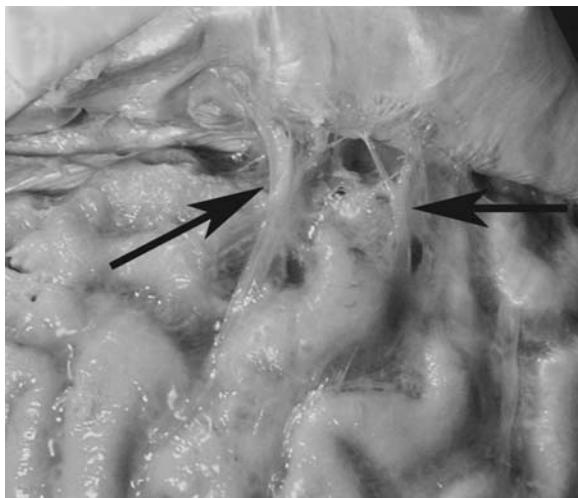


Fig. 22. Bridging veins (arrows) arising from the cerebral cortex are seen entering the superior sagittal sinus within the dura mater. After death, the arachnoid collapses on the surface of the bridging veins and the cerebral cortex revealing the subdural space that is a potential space in the living. Tearing of the bridging veins causes a subdural hematoma.

way of the *inferior anastomotic vein* (Labbe's vein; Fig. 21). The *inferior cerebral veins* are located on the anterior surface of the cerebral cortex. The more anterior branches drain into the superior cerebral veins, and the more posterior branches empty into the middle cerebral vein. The internal venous drainage of the cerebral cortex is conducted by the *great cerebral vein* (Galen's vein) and begins by its tributaries—the internal cerebral veins that drain the deep regions of the cerebral cortex. These in turn are formed by the thalamostriate and choroid veins. The great cerebral vein joins with the inferior sagittal sinus to empty into the straight sinus of the dural sinuses. The cerebellum is drained by superior and inferior cerebellar veins on its surface. Each superior cerebellar vein passes anteriorly and medially to enter the straight sinus, and each inferior cerebellar vein drains into the superior petrosal sinus or transverse sinus. The brain stem is drained by a venous plexus composed of anterior vessels that drain into the vertebral venous plexus, the lateral venous plexus that drains into the inferior petrosal sinus, and a posterior plexus that drains into the great cerebral vein or straight sinus.

Veins of the face, orbit, scalp, and bones of the skull communicate directly with the dural venous sinuses through named vessels (e.g., the superior ophthalmic vein), as well as through a series of *emissary veins* that enter the cranial vault through small foramina. These valveless, venous communications

between superficial structures and the dural venous sinuses are clinically important because they provide a route for infections originating outside of the cranial vault to either thrombose a dural venous sinus and/or enter into the brain.

3.8. Ventricular System

The ventricular system of the brain is a series of cavities connected to one another throughout the different brain regions. Its complex adult shape develops from the straight, centrally located neurocele of the embryonic neural tube (Fig. 23). The two large C-shaped lateral ventricles are formed as the prosencephalic vesicles expand laterally and carry along the neurocele lumen. The ventricular system contains CSF produced by the *choroid plexus* that is also located in the ventricles (Fig. 24A–C). The ventricular system opens into the subarachnoid space, allowing CSF to surround the brain and spinal cord for support and protection. Indeed, the brain would sag under its own weight if not buoyed by CSF.

A *lateral ventricle* is contained within each cerebral hemisphere, and these are separated from each other along the midline by the thin *septum pellucidum*. Each



Fig. 23. A cast of the ventricular system of the human brain. The ventricular system was filled with latex and the brain tissue removed to reveal the anatomy of the ventricular system. The two lateral ventricles are each composed of the frontal pole (F), body (B), posterior pole (P), and inferior pole (I). The three poles (also called horns) are found in the frontal, occipital, and temporal lobes, respectively. The enlarged region where the body and posterior and inferior poles meet is called the antrum (A). The lateral ventricles communicate with the midline third ventricle (III) via the interventricular foramen (InVF). The hole in the cast of the third ventricle is caused by the presence of the mass intermedia of the thalamus. The cerebral aqueduct (CA), the narrowest portion of the ventricular system, allows communication between the third ventricle and the fourth ventricle (IV).

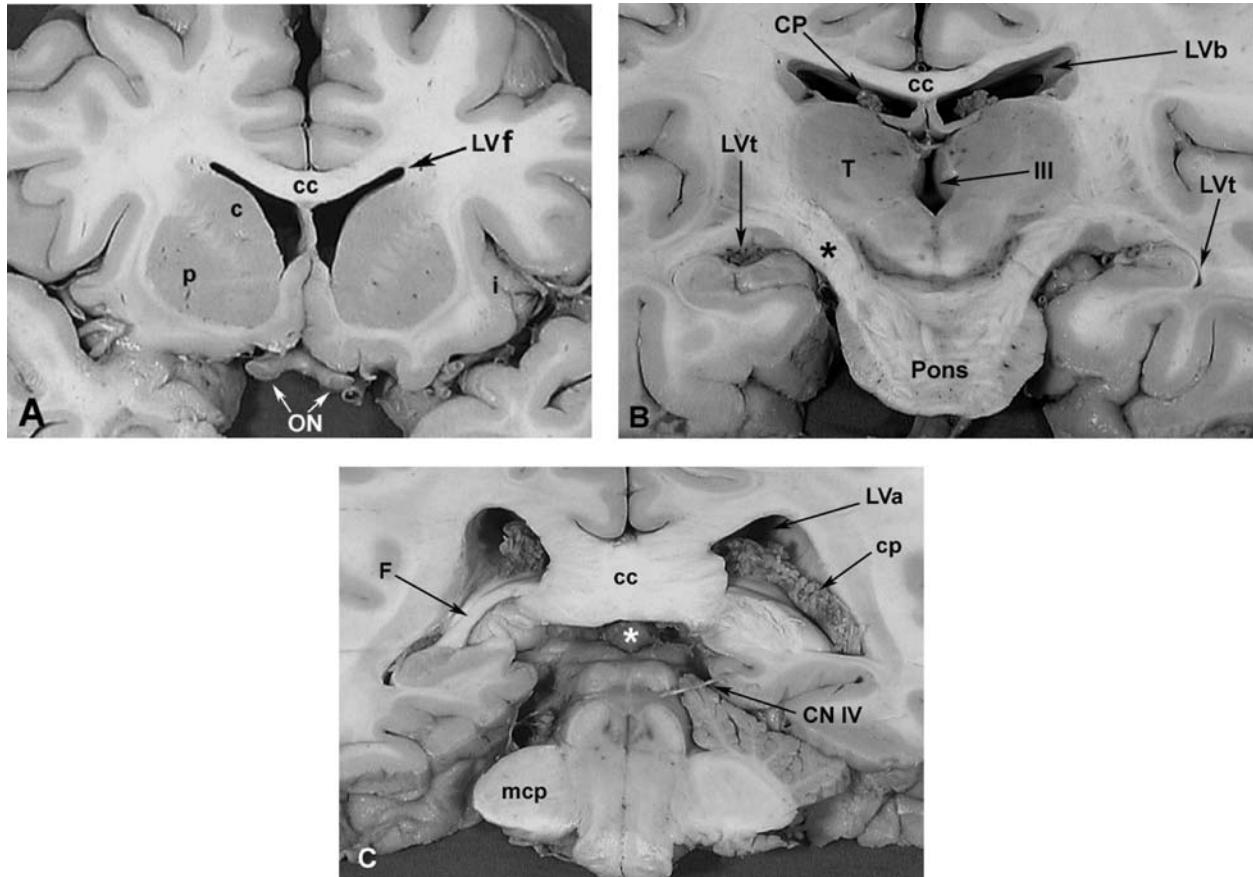


Fig. 24. Ventricular system within cerebral coronal sections. **(A)** The frontal pole of each lateral ventricle (LVf) is deep to the corpus callosum (cc). The septum pellucidum separates the lateral ventricles. The caudate (c) and putamen (p) of the basal ganglia are visible, as are the optic nerves (ON) and the insular (i) cortex. **(B)** The body of the lateral ventricles (LVb) and the third ventricle (III) are present at the level of the thalamus (T). The temporal cortex contains the temporal pole of the lateral ventricles (LVt). Choroid plexus (CP) is present in the lateral ventricles. The asterisk lies on the cerebral peduncle of the midbrain. **(C)** The atria of the lateral ventricles (LVa) are visible at the level of the splenium of the corpus callosum (cc). Each atrium contains choroid plexus (cp) and can be seen curving inferiorly to enter the temporal pole of the lateral ventricle. The fornix (F) is visible arising from the hippocampal formation. The trochlear nerve (CN IV) arises from the posterior midbrain just inferior to the inferior colliculus. The asterisk lies on the pineal gland of the diencephalon. The middle cerebellar peduncle (mcp) of the pons is seen.

lateral ventricle is subdivided into four interconnected regions (Fig. 23 and Fig. 24). Its *frontal horn*, located in the frontal cortex, is continuous with the *body* located in both the frontal cortex and parietal cortex. Posteriorly, the body curves anterolaterally into the temporal cortex as the *inferior horn*. The *posterior horn* extends from the body into the occipital cortex as it begins to curve into the inferior horn. The expanded region of the lateral ventricle where the body, inferior and posterior horns unite is called the *antrum*. Each lateral ventricle communicates with the remainder of the ventricular system by an *interventricular foramen (of Monro)*, which is located at the midrostral end of the lateral ventricles anterior to the fornix (Fig. 13 and Fig. 23). The adult

interventricular foramen is the site where an embryonic prosencephalic vesicle started to bulge laterally and maintains the patent communication to the midline neurocele (Fig. 23).

The interventricular foramen connects each lateral ventricle to the thin, midline *third ventricle* located between the medial borders of the paired diencephalic structures. The *cerebral aqueduct*, located in the tectum of the midbrain, is the narrowest portion of the ventricular system and connects the third ventricle with the fourth ventricle. During development, the cerebral aqueduct can become either stenotic or blocked. If this occurs, then the continual production of CSF, via the choroid plexus in the lateral ventricles and the third ventricle, results in a *noncommunicating*

hydrocephalus where the lateral and third ventricles enlarge in size due to the accumulation of CSF that is unable to exit the ventricular system via foramina in the fourth ventricle.

The *fourth ventricle* is a rhomboid-shaped space located between the cerebellum posteriorly and the pons and medulla oblongata anteriorly (Fig. 9, Fig. 12, Fig. 13, and Fig. 23). It is continuous with the central canal of the spinal cord that is only intermittently patent along its length. More importantly, the roof of the fourth ventricle opens into the subarachnoid space by the single median foramen (of Magendie) and the paired lateral foramina (of Luschka). These three foramina allow egress of CSF from the ventricular system into the subarachnoid space. The fourth ventricle also contains choroid plexus. The entire ventricular system is lined by a single layer of ependymal cells, except where the choroid plexus is located. Ependymal cells differentiate from the proliferative cells that line the neurocèle once they are finished giving rise to neuroblasts and gliablasts.

The subarachnoid space is enlarged in places, allowing for accumulation of CSF between the arachnoid membrane and pia mater to help cushion and protect the brain from the surrounding skull. The posterior *cerebellomedullary cistern*, the site of drainage of the lateral and median foramina openings from the fourth ventricle, is the largest of the subarachnoid spaces. It is located between the medulla and cerebellum posteriorly and is continuous with the subarachnoid space surrounding the dorsal side of the spinal cord. The *quadrigeminal cistern* (cistern of the great cerebral vein) is located posteriorly in the interval between the splenium of the corpus callosum and the superior surface of the cerebellum. It is continuous with the cerebellomedullary cistern caudally. The *pontine cistern* is located on the anterior surface of the pons between it and the more caudal medulla oblongata. It is continuous caudally with the subarachnoid space surrounding the ventral side of the spinal cord and the interpeduncular cistern more rostrally. The *interpeduncular cistern*, located between the cerebral peduncles, contains the origin of the oculomotor nerves (CN III). This region is also described as the interpeduncular fossa on specimens that have their meninges removed. The *chiasmatic cistern* is the rostral continuation of the interpeduncular cistern. It is located anterior and inferior to the optic chiasm on the anterior surface of the brain. This cistern is in turn continuous laterally with the subarachnoid space in the cranial fossa.

The CSF located in the ventricular system is produced by the *choroid plexus* found in the lateral ventricles and the third and fourth ventricles; it is absent in the cerebral aqueduct (Fig. 23 and Fig. 24A–C). The choroid plexus is a complex vascular system composed of pia mater covered by choroid epithelial cells that become invaginated by numerous capillary tufts. During development, portions of the alar plates of the rostral neural tube swing laterally. During this process, the pia mater on the CNS surface comes in contact with the ependymal cells that line the developing ventricular system. When this contact occurs, the ependymal cells differentiate into choroid epithelial cells and become invaginated with capillaries to form the choroid plexus. Production of CSF by the choroid plexus results in circulation beginning in the lateral ventricles, through the third ventricle into the fourth ventricle, and out into the subarachnoid space through the median and lateral foraminae. Once in the subarachnoid space, the CSF circulates throughout the subarachnoid space surrounding the brain and spinal cord and is then reabsorbed into the venous system from the subarachnoid space at the superior sagittal sinus by tuft-like protrusions, the *arachnoid granulations* of the arachnoid membrane (Fig. 25). Venous blood has a lower pressure than does CSF, so CSF naturally flows from the subarachnoid space into the superior sagittal sinus. If the arachnoid granulations become blocked by red blood cells (due to a subarachnoid hemorrhage) or bacteria, then the flow of CSF out of the subarachnoid space is compromised and a *communicating*

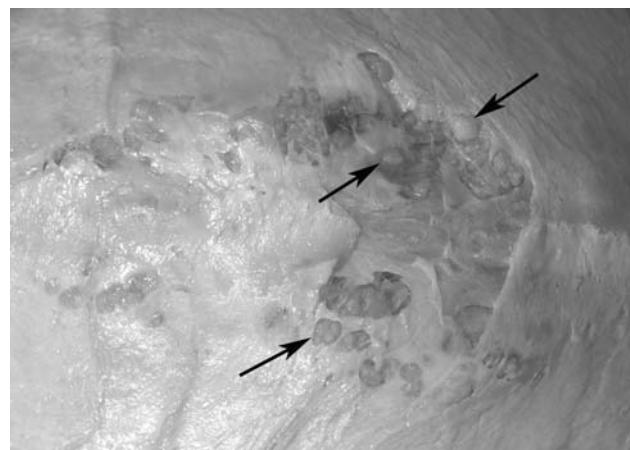


Fig. 25. Arachnoid granulations. When the calvaria is removed, most of the periosteal layer of the dura mater peels away from the inner surface of the skull. In this specimen, some of the periosteal layer remained in the skull and opened the lumen of the superior sagittal sinus to reveal arachnoid granulations (arrows).

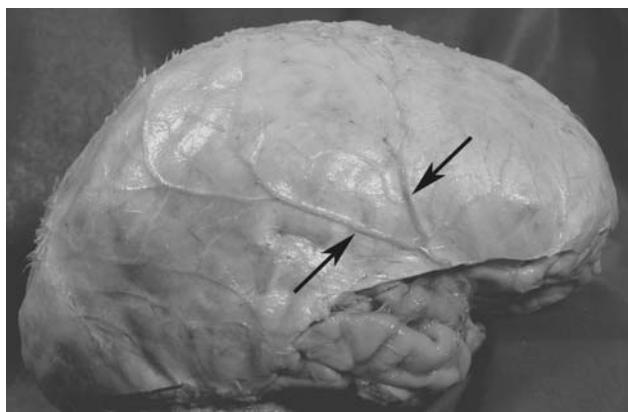


Fig. 26. A lateral view of the brain with most of the dura mater intact. Portions of the frontal and temporal cortices are seen inferior to the cut edge of the dura mater. The middle meningeal artery (arrows), a major supplier of blood to the meninges, can be torn with a blow to the side of the head. The subsequent arterial bleed forms an epidural hematoma.

hydrocephalus can develop. In this instance, the CSF can exit the ventricular system but cannot be removed from the subarachnoid space into the venous system.

3.9. Meninges of the Brain

The dura mater of the cranial cavity is composed of a superficial *periosteal layer* and a deeper *meningeal layer* (Fig. 26). The meningeal layer is continuous with the meningeal layer of the spinal cord in the vertebral canal at the foramen magnum, and the periosteal layer ends at the foramen magnum. Both layers in the cranial cavity are adherent to each other except in regions where the venous dural sinuses force them apart. The arachnoid membrane lies deep to the dura mater and is continuous with the arachnoid membrane of the vertebral canal. Like the arachnoid membrane in the vertebral canal, it has fibrous extensions down to the pia mater and is pushed against the inner surface of the dura mater by CSF pressure. The pia mater in the cranial cavity follows the surface of the brain and is found in all sulci and fissures of the brain. Within the lateral ventricles, the third ventricle, and the rostral end of the fourth ventricle, the pia mater invaginates into the ventricular spaces to contribute to the formation of the choroid plexus. The epidural space associated with the spinal cord is not present as a true space in the cranial cavity. The cranium has two potential spaces, the epidural and subdural; they are present only when bleeding forms hematomas that force them open. The subarachnoid space is filled with CSF and surrounds the brain and allows it to float in the cranial cavity. It is continuous with the

subarachnoid space of the vertebral canal surrounding the spinal cord. A number of enlarged regions of the subarachnoid space are present on the anterior and posterior sides of the brain and have already been described.

Extensions of the meningeal layer of the dura mater are located in specific regions within the cranial vault and contain dural sinuses used to drain blood away from the brain. The first is the vertically oriented *falk cerebri*, which extends into the longitudinal fissure between the two halves of the cerebral cortex. The second is the horizontally oriented *tentorium cerebelli*, attached from the occipital bone posteriorly to the petrosal ridge of the temporal bone anterolaterally. It is located between the inferior surface of the occipital cortex and the superior surface of the cerebellum. In the midline, the tentorium cerebelli does not attach to any bony structure forming an opening, the *tentorial incisure*, which allows the brain stem to pass from the posterior cranial fossa into the middle cranial fossa. The third is the small, vertically oriented *falk cerebelli* located between the two cerebellar cortices. The fourth is the horizontal *diaphragma sellae* that covers the sella turcica of the sphenoid bone and surrounds the infundibulum of the pituitary.

All of these dural specializations are stout immovable structures. Because of this, swelling of a portion of the brain or a space occupying lesion within the skull cannot push these dural specializations aside, therefore the soft brain matter herniates around these structures with severe consequences. For example, a tumor in the temporal lobe can cause an *uncal herniation* where the uncus on the parahippocampal gyrus is pushed over the free edge of the tentorium cerebelli. This compresses the oculomotor nerve, resulting in a third nerve palsy, and compresses the midbrain whose integrity is vital for maintaining consciousness. In a similar fashion, a mass in the cerebellum cannot expand superiorly because of the tentorium cerebelli, therefore the cerebellar tonsils herniate through the foramen magnum (*tonsillar herniation*) causing compression of the medulla that is rapidly fatal because the medulla contains cardiovascular and respiratory control centers. The diaphragma sellae is also of clinical significance when a severe blow to the head causes the brain to move within the skull. If the movement is large enough, the hypothalamic infundibulum can be sheared off the pituitary that is located deep to the diaphragma sellae. Although anterior pituitary function usually remains intact, the disruption of the axonal connection between the hypothalamus and the posterior

pituitary results in a loss of vasopressin (also called antidiuretic hormone) release into the bloodstream with the subsequent development of diabetes insipidus.

Dural sinuses are modified venous structures lined with endothelium contained within the dura mater of the cranial cavity (Fig. 21). The *superior sagittal sinus* is present along the superior edge of the falx cerebri, where it is bounded by both the periosteal dura mater and the meningeal dural mater. It begins rostrally at the crista galli of the ethmoid bone and continues posteriorly to empty into the *confluens* of the sinuses located on the occipital bone. The superior sagittal sinus receives the CSF drainage by way of arachnoid villi that extend into its lumen. Along the inferior free edge of the falx cerebri, the smaller *inferior sagittal sinus* is contained entirely within the meningeal layer of the dura mater. It also begins at the crista galli and empties into the straight sinus at the anterior border of the junction of the falx cerebri and tentorium cerebelli. The *straight sinus* begins at the anterior border of the tentorium cerebelli, where it receives blood from the great cerebral vein (of Galen) and the straight sinus and empties posteriorly into the *confluens* of the sinuses. The falx cerebelli contains the occipital sinus along its posterior free edge. The *confluens of the sinuses*, located on the internal occipital protuberance, communicates with the superior sagittal sinus, straight sinus, occipital sinus, and transverse sinuses. The *transverse sinuses*, formed between the periosteal and meningeal layers of dura mater, are located along the posterior edges of the tentorium cerebelli, where the sinus contacts the occipital and parietal bones. These drain blood from the confluence of the sinuses and the superior sagittal sinus anteriorly. The *sigmoid sinuses* are continuations of the transverse sinuses as they leave the tentorium cerebelli and take a medial and inferior serpentine course along the inner surface of the temporal bone to empty into the jugular foramen where the internal jugular vein originates. The *cavernous sinuses* are located deep to the diaphragma sellae on either side of the sella turcica of the sphenoid bone. They communicate with one another by *intercavernous branches*. The cavernous sinuses are unique to other dural sinuses because they contain the internal carotid artery and abducens nerve (CN VI) within their lumen and the ophthalmic and maxillary divisions of the trigeminal nerve (CN V) and the oculomotor nerve (CN III) in the lateral wall of the sinus. Anteriorly, the cavernous sinus communicates with superficial veins of the face, ophthalmic veins, and the

pterygoid plexus. Posteriorly, the cavernous sinus communicates with the inferior petrosal sinus, superior petrosal sinus, and the basilar plexus of veins. The *superior petrosal sinus*, located within the anterolateral border of the tentorium cerebelli, is located along the superior border of the petrous ridge of the temporal bone. It begins at the cavernous sinus and joins with the transverse sinus to empty into the sagittal sinus. The *inferior petrosal sinus* begins at the cavernous sinus and follows the temporo-occipital suture to the jugular foramen.

One of the major blood supplies to the dura is the middle meningeal artery (Fig. 26). This artery is contained within the dura mater once it enters the skull through the foramen spinosum of the sphenoid bone. This artery is of great clinical significance. The course of the middle meningeal artery forms a depression along the inner surface of the cranial vault, and often bone may completely surround small segments of the artery. Blows to the side of the head that fracture the skull in the region of the pterion can cause a laceration of the middle meningeal artery that results in high-pressure arterial bleeding. The blood accumulates between cranial vault and dura mater forming an epidural hematoma. A rapidly expanding epidural hematoma constitutes a medical emergency because the accumulated blood forms a space-occupying lesion causing herniation of the cerebral cortex.

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3

Ion Channels, Transporters, and Electrical Signaling

Stanko S. Stojilkovic

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Abbreviations: ASICs: acid-sensitive ion channels, Ca_v channels: voltage-gated Ca^{2+} channels, CCCs: calcium-chloride co-transporters, ClC: voltage-gated chloride channels, CNG channels: cyclic nucleotide-gated channels, CNS: central nervous system, CRAC channels: calcium release-activated channels, ENaC: epithelial sodium channels, ER: endoplasmic reticulum, GABA: γ -amino butyric acid, GluRs: glutamate receptors, HCN channels: hyperpolarization-activated cyclic nucleotide-modulated channels, HVA: high voltage activated, IP₃: inositol 1,4,5-trisphosphate, IP₃R: IP₃ receptors, K_{Ca}: voltage-gated potassium channels, K_{ir}: inwardly rectifying potassium channels, K_v: voltage-gated potassium channels, K_{2P}: 2P loop potassium channels, LVA: low voltage activated, nAChR: nicotinic acetylcholine receptor, Na_v: voltage-gated sodium channels, NCX: sodium-calcium exchanger, PKA: cAMP-regulated protein kinase, PKC: protein kinase C,PKG: cGMP-regulated protein kinase, P2X: purinergic receptor channels, RyRs: ryanodine receptors, TM: transmembrane domain, TRP: transient receptor potential, TTX: tetrodotoxin.

OVERVIEW

Membranes in all cell types regulate the extracellular and intracellular ionic environment. In neurons and other excitable cells, regulation of the ionic environment is also crucial for the development and maintenance of the specific signaling pathway for these cells, known as the *electrical signaling system*. This system is composed of two basic elements: (1) a lipid bimolecular diffusion barrier, termed *lipid bilayer*, which separates cells from their environment, and (2) two classes of macromolecule proteins, known as *ion channels* and *active transporters*, which regulate the movement and distribution of ions across the lipid barrier in the plasma membrane. Resting neurons generate a negative potential, termed the *resting membrane potential*. The primary electrical signal generated by neurons is called the *action potential*, which abolishes the negative resting potentials and makes the transmembrane potential transiently positive. The main distinction between these two processes is that the membrane potential is generated by channels that are active at rest, whereas action potentials are generated by channels that open when the cell is electrically active. A negative membrane resting potential results predominately from a net efflux of K^+ across membrane. Generation of action potential is achieved by a rapid raise in Na^+ and/or Ca^{2+} permeability, leading to *depolarization* and *inverse polarization* of cells, whereas a slower and prolonged rise in K^+ permeability accounts for *repolarization* of cells to their usual negative resting level. The same mechanism also is responsible for propagation of action potentials along the length of axons. Changes in permeability for Ca^{2+} , on the other hand, provide an effective mechanism for transfer of electrical signals from cell to cell by initiating the release of neurotransmitters at synapses. *Ion channels* are the membrane proteins that give rise to selective permeability, whereas *active transporters* create and maintain ion gradients. Thus, from the perspective of electrical signaling, ion channels and active transporters are complementary. Some of the ion channels are able to sense the electrical potential across the plasma membrane and are called *voltage-gated channels*. These channels have a pore that opens and/or closes in response to changes in the membrane potential. Other channels are gated by extracellular chemical signals and are called *extracellular ligand-gated channels*. Among transporters, the most important is the Na^+ pump, which regulates the intracellular concentrations of both Na^+ and K^+ and requires ATP for its action. The concentration gradients of Ca^{2+} , Cl^- ,

and H^+ are also achieved by co-transporters specific for these ions and usually sodium ion. Neurons, like other cell types, also signal through nonelectrical plasma membrane-dependent mechanisms, independently of ions and proteins responsible for ion transport. This *receptor-mediated signaling* frequently interacts with the electrical signaling system.

1. RESTING MEMBRANE POTENTIALS

1.1. Lipid Bilayer Separates Cells from Their Environment

Phospholipids and several other types of lipids contribute to the membrane structure. The major cell-membrane lipids consist of a hydrophilic head, commonly a glycerophosphoryl ester, and a hydrocarbon tail, usually containing two hydrophobic fatty acids. When exposed to water, phospholipid molecules usually orient with their two hydrophobic hydrocarbon tails to one another and their hydrophilic polar or charged head groups adjacent to the water molecules. In the cell membrane, tails orient back-to-back so that the hydrophobic lipid tails of one layer face the hydrophobic tails of the other, whereas the hydrophilic heads point outward away from the middle of the membrane. Such a double-layered structure is known as a *lipid bilayer* (Fig. 1). Detergents transform lipid bilayers into water-soluble micelles, whereas cholesterol stabilizes bilayers.

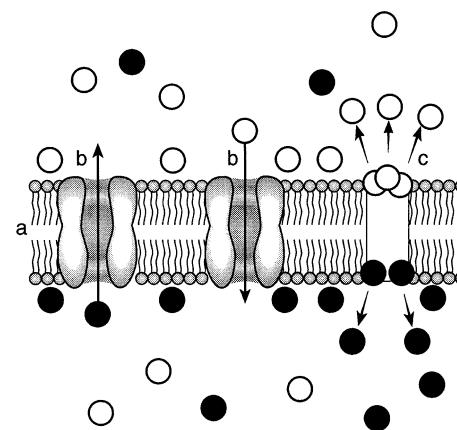


Fig. 1. Two essential components of neuronal-cell membrane responsible for electrical signaling are (a) the phospholipid bilayer and two classes of macromolecular proteins, (b) ion channels and (c) ion transporters. (b) A difference in concentration of an ion across the plasma membrane will result in a net movement through channels away from the side of higher concentration to the side of lower concentration by passive transport known as diffusion. (c) An ion pump is working in the opposite direction of the concentration gradient.

The bilayer structure serves several important functions in intracellular and intercellular signaling. (1) The hydrophobic tails of the lipid molecules tend to be chemically incompatible with water-soluble substances containing inorganic ions. As a result, the lipid bilayer serves as a barrier to the movement of ions across the membrane, effectively separating the intracellular and extracellular ionic compartments. One class of bilayer lipids provides substrates for signal transduction enzymes, leading to the generation of several intracellular signaling molecules. (2) Separation of intracellular and extracellular conducting solutions is provided by an extremely thin hydrophobic, insulating layer (1.5 to 3 nm). This forms a significant electrical capacitor, or storage of electrochemical energy that drives the signaling process. (3) The protein components of the membranes, including carriers and ion channels, are embedded in the lipid bilayer and are oriented and grouped in a manner that serves their respective functions. Lipid membranes are also critical in forming organelles, such as the endoplasmic reticulum (ER), which is composed of rough ER or Nissl substance, and smooth ER. The latter contains ion transporters and channels and provides an additional mechanism for intracellular signaling in neurons.

1.2. Ion Pumps and Ion Channels Transport Ions in Opposite Directions

The lipid bilayer retains vital cell compartments but also prevents the exchange of ionized substrates between the cell and its environment, which is critical not only for electrical signaling but also for cell metabolism in general. This function is mediated by specific ion-transporter mechanisms. Ion carriers, including the $\text{Na}^+ \text{-K}^+$ pump, the Ca^{2+} pump, $\text{Na}^+ \text{-Ca}^{2+}$ exchanger, $\text{Cl}^- \text{-HCO}_3^-$ exchanger, and glucose transporters, are macromolecules fixed in the membrane and their transporter-binding sites are exposed alternatively to the intracellular and extracellular media or luminal and intracellular face of the ER membrane. Ion pumps require adenosine triphosphate (ATP) as the source of energy to move ions from a side of low concentration to a side of high concentration. For example, Na^+ and K^+ are moved across the membrane by the $\text{Na}^+ \text{-K}^+$ exchange pump in opposite directions. In every pump cycle, three Na^+ are moved from cytosol to the extracellular fluid, two K^+ are moved from the extracellular fluid into the cytosol, and one ATP is required to make this movement (Fig. 2). This process has been observed in nearly all cell types that have been studied. The

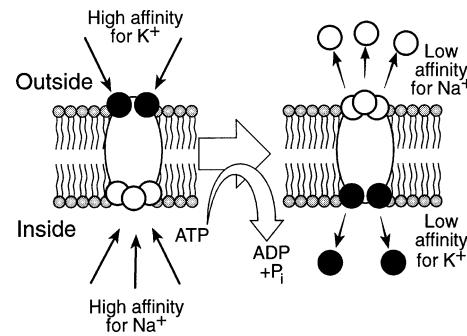


Fig. 2. A schematic representation of the sodium-potassium pump transporting Na^+ and K^+ across the membrane against their electrochemical gradient. In one conformation state, the carrier binds 3Na^+ on the intracellular surface and 2K^+ on the extracellular surface, both with high affinity (left panel). Following a series of conformation changes driven by ATP hydrolysis, the carrier releases Na^+ to the extracellular fluid and K^+ on the cytoplasmic surface (right panel). In that conformation stage, the carrier has a low affinity for Na^+ and K^+ , limiting the reverse transports of ions. The activity of the sodium-potassium pump is critical in establishing and maintaining the resting potential.

ATP-dependent Ca^{2+} transporters are expressed in both plasma membrane and ER membrane and pump Ca^{2+} from cytosol to the extracellular fluid and the ER fluid, respectively. Together with $\text{Na}^+ \text{-Ca}^{2+}$ antiporters, these pumps maintain low concentrations of intracellular free Ca^{2+} in resting cells (about 100 nM). Thus, by working against the concentration gradient, an ion pump is building up a high concentration of ions on one side of the membrane, and such ion gradients are required for the most basic neuronal functions.

However, excitation and electrical signaling *per se* are carrier-independent and are dependent on movements of ions through ion channels. This movement is determined by ion gradient across the cell membrane and does not require energy. Ion channels have water-filled pores through the plasma membrane, which are permeable for ions and in some cases for small molecules as well. An ion channel is usually built of several subunits, each of which is composed of helical strands that form transmembrane (TM) domains (shown in Fig. 3 as cylinders). These domains are connected by extracellular and intracellular chains of amino acids (shown in Fig. 3 as full lines). The role of ion channels is to set the permeability of the membrane. One group of channels passes cations (positively charged ions), and the other group passes anions (negatively charged ions). Some channels are nonspecific for cations, but most channels are ion-selective (e.g., they allow the passage of a specific ion). This

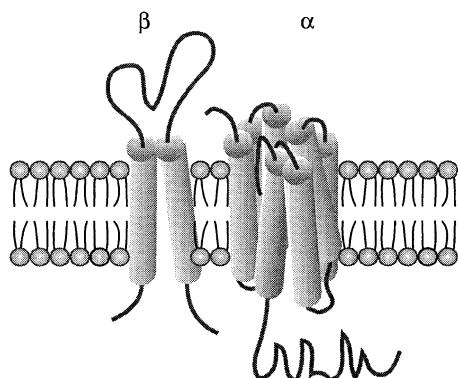


Fig. 3. Ion channels are expressed in all living cells and have numerous functions. This includes establishing a resting membrane potential, generating action potentials and action potential-dependent intracellular calcium signals, and controlling cell volume and net flow of ions and fluids. Ion channels can have different transmembrane (TM) topologies and are usually composed of several subunits. Subunits have different number of TM domains connected by intracellular and extracellular chains.

property had been used to name the channels (e.g., Na^+ , K^+ , Ca^{2+} channels). Some channels are continuously open to the flow of ions and are termed *leakage channels*. Others are transiently open to ion fluxes and are known as *gated channels*. Closing of channel is a conformation change that may involve a decrease in diameter of the pore or the movement of terminal parts of protein to block the pore to the flow of ions. A part of the molecule that moves to occlude or open the channel is termed a *gate*. There are five major groups of channels. (1) *Voltage-gated channels* open or close in response to changes in electrical potential across the cell membrane. (2) *Ligand-gated channels* require a binding of a particular signaling molecule to open or close. (3) *The stretch-sensitive channels* open or close in response to a mechanical force. (4) *Intracellular channels* are expressed in the ER and nuclear membranes. (5) Connexins and pannexins form gap junctions, which operate as *intercellular channels*.

1.3. Signaling Proteins Other than Channels and Transporters

Signaling proteins of the cell membrane include three groups of proteins. (1) *Adhesion* and *anchor* proteins help bind one cell to another and fasten the membrane to an internal network of proteins, respectively. (2) *Receptors* are proteins that bind the extracellular messengers and respond to this by generating intracellular messengers. Each receptor is

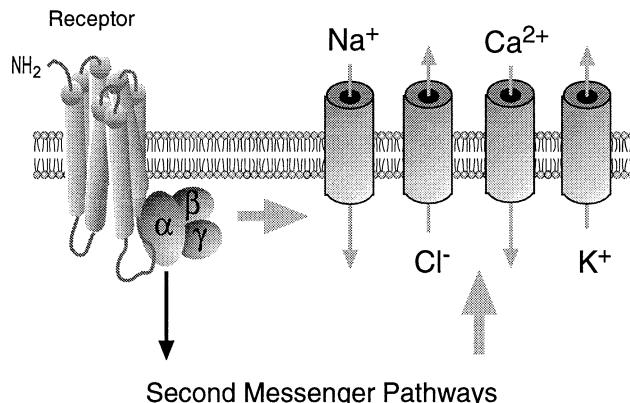


Fig. 4. Seven TM-domain receptors are coupled to heterotrimeric G proteins, leading to the activation of several intracellular signaling pathways, including phospholipase C and adenylyl cyclase-dependent pathways. Receptor-induced dissociation of G- α and β/γ dimers provides a mechanism for regulation of ion-channel gating by these subunits (membrane-delimited pathway). The gating of channels is also controlled in a second-messenger-dependent manner.

specific for only one type of signaling molecule, as well as to its close chemical analogs. In neuronal cells, they are usually concentrated on the postsynaptic membranes. (3) Receptors transduce signaling by activating a chain reaction or a cascade of events, usually by activating membrane-bound enzymes. The first protein in the cascade is G protein, which in turn activates other membrane-bound proteins, including adenylyl cyclase, phospholipases C and D, and phosphodiesterases. This also leads to the generation of *intracellular messengers*, such as cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), inositol 1,4,5-trisphosphate (IP_3), diacylglycerol (DAG), and phosphatidic acid, as well as activation of kinases, including protein kinase A (PKA), protein kinase C (PKC), and protein kinase G (PKG) (see Chapter 5). These intracellular messengers have numerous functions, including the control of ion-channel conductivity (Fig. 4).

1.4. Ion Concentrations Differ Across the Cell Membrane

The concentrations of various ions in the two conducting solutions, the intracellular and extracellular, are different. All living cells maintain differential ion concentrations across the cell membrane. Table 1 lists the concentrations of major ions in a mammalian cell model. Na^+ , Ca^{2+} , and Cl^- are predominately found extracellularly, whereas K^+ and organic ions are

Table 1
Distribution of Free Ion Concentrations and Equilibrium Potentials for a Mammalian Cell Model

Extracellular ion	Intracellular concentration (mM)	Equilibrium concentration (mM)	Potential (mV)
Sodium	145	12	+67
Potassium	5	150	-91
Calcium	1.5	0.0001	+128
Chloride	125	5	-86

concentrated intracellularly. The *concentration gradient* across the membrane has two major functional consequences for excitable cells: (1) It provides the electrochemical energy to drive signaling. Na^+ and K^+ are essential for this process, and Ca^{2+} concentration gradient also helps to drive the signaling. (2) Ca^{2+} influx through plasma-membrane channels also serves as an intracellular messenger to activate many intracellular biochemical processes, including synaptic transmission. The concentration gradient for Ca^{2+} across the ER membrane resembles that of the plasma membrane, and the release of this ion from the luminal side plays a critical role in intracellular signaling of excitable and nonexcitable cells.

1.5. Every Neuron Has a Membrane Potential

There is a balance of electrical charge within the cytosol and extracellular fluid compartment, termed *macroscopic electroneutrality*. On the other hand, there is a difference in electrical potential across the membrane of each cell. Experimentally, this difference can be measured by placing a glass microelectrode into the cytoplasm and the other electrode in external fluid (Fig. 5). A typical microelectrode is a piece of glass tubing with a very fine opening of less than 1 μm diameter. The electrode is filled with

a good electrical conductor and is connected to a voltmeter or oscilloscope the transmembrane voltage. In excitable cells, a steady electrical potential difference of about -50 to -70 mV is detected. In nonexcitable cells, the potential difference is smaller. By convention, the sign of potential difference indicates that the inside of the cell is electrically negative with respect to the outside. The membrane potential is always given as that of intracellular compartment relative to the extracellular compartment. This difference is termed the *membrane potential*. Nearly every aspect of electrical signaling in excitable cells depends in some way on the membrane potential. Signals that decrease membrane potential are *depolarizing* and those that increase the membrane potential are *hyperpolarizing*.

1.6. The Membrane Potential as Cellular Force

Separation of charges of opposite sign, like those by plasma membrane, provides the basis for electrical phenomena. As in physics, we can talk about the *potential difference*, measured in volts (V), *current*, measured in amperes (A), *conductance*, measured in siemens (S), and *resistance*, which is measured in ohms (Ω). As stated previously, in cells the potential difference is generated by unequal cations and anions on two sides of the membrane. When positive and negative electrodes are placed in an ion solution, cations will flow toward the negative pole and anions toward the positive pole, and both carry electrical current toward the negative pole. The size of current is determined by the potential difference between the electrodes and the electrical conductance of the solutions between electrodes. The conductance in salt water depends on salt concentrations and mobility of ions. Resistance is the reciprocal of conductance. Furthermore, capacitance (C) is a measure of how much charge must be transferred from one conductor to another, and is defined as:

$$C = Q/E \quad (1)$$

where Q is the charge and E is voltage difference across the conductor. The unit of capacitance is the farad (F). Because electrical capacitance is inversely related to the thickness of the insulating region separating two conductors, the 1.5- to 3-nm lipid barrier makes a very efficient capacitors, of the order $1 \mu\text{F}/\text{cm}^2$ of membrane surface.

The resistance of the cell membrane indicates how difficult it is for ions to move through. If there are few open channels for an ion, the membrane has a high resistance and a low conductance for that ion. In

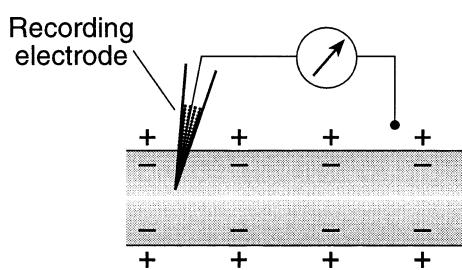


Fig. 5. The intracellular recording of membrane potential in an axon using a fine glass microelectrode. The circle with arrow indicates a combined amplifier and voltage-measuring device.

general, the conductance of a particular membrane depends on several factors: (1) the density of the channels; (2) the number of channels that are open; and (3) the maintenance of ion concentrations. The first two factors are the most significant for conductance across the membrane, whereas the relevance of ion concentrations depends on the volume of the cells and myelination of the membrane (see Section 2.5). For example, in a nonmyelinated cell 25 μm in diameter, the amount of charge required to be moved across cell membranes to support physiologic changes in membrane potential is usually accompanied with negligible changes in the ion concentrations across the membrane (1 of about 200,000). However, in small cells and in axons, depletion and recovery of ions in cytoplasm during electrical signaling compared with the stored-up ions is much higher and could affect electrical signaling during the sustained activation.

1.7. Membrane Potential and Ion Movements

In neurons that are electrically inactive (quiescent), membrane potential is in a steady state and is termed *resting potential*. This potential results from a separation of positive and negative charges across the plasma membrane. This means that the movement of ions across the membrane in one direction is balanced by the movement of the same number of ions in the opposite direction. In general, three factors contribute to the movement of ions: concentration gradient, electrical gradient, and transporter activity. (1) A difference in concentration of the ion on the two sides of the membrane should result in *diffusion*, a net movement of ions away from a region of greater concentrations toward a region of lesser concentration. Thus, if the membrane is permeable for ions, they will move down its concentration. The net transfer is proportional to the concentration difference across the cell membrane, does not require energy, and is termed a *passive transport*. (2) Electrical potential differences across the cell membrane also influence ion movement. Cations in the pore of channels will be moved toward the negative interior of cells, whereas anions will be moved toward the positive exterior of cells, and this transport does not require energy. (3) Pumps also participate in the transport of ions across the membrane, but the energy stored in ATP is usually required and ions are moved against their concentration gradients (*active transport*).

The intact membrane is essential for the maintenance of resting potential. In cells with a damaged membrane, a resting potential rapidly declines to zero. Also, if channels are continuously open and

conduct all ions, passive transports will bring the resting potential to zero because of diffusion of ions. Furthermore, cells have to be bathed in medium containing ion concentrations comparable with that of *in vivo* extracellular medium. For example, with an increase in external concentrations of K^+ from the physiologic 5 mM to 50 mM by substituting NaCl with KCl in extracellular medium, the resting potential will decline. In other word, the cell will depolarize. This simple experimental procedure indicates that concentration gradient for K^+ is important in controlling resting potential and is frequently used to activate voltage-gated Ca^{2+} entry. Finally, inhibition of metabolic activity attenuates the carrier activity, leading to gradual (over hours) decline of resting potential to zero. These observations indicate that the generation and maintenance of resting potential requires (1) unequal distribution of ions across the membrane; (2) the selective permeability of ion channels and their ability to close; (3) the energy-dependent transport, which is not critical in rapid responses, but is required to maintain a resting potential over a long period of time.

1.8. Single-Ion Electrochemical Equilibrium

To understand the role of ion gradients and selective permeability in generating a negative membrane potential, we will first discuss a hypothetical cell model that contains sodium chloride extracellularly, potassium aspartate intracellularly, and a plasma membrane expressing K^+ channels only. Initially, both intracellular and extracellular sides will be electrically neutral, because of the equal numbers of cations and anions. If the cell contains open channels that are selective for K^+ , this ion will move through the channels down its concentration gradient with tendency to equilibrate its concentration on both sides. Because K^+ is charged, however, the diffusion of each K^+ makes the inside slightly more negative relative to outside, leading to the generation of a potential difference, as well as the generation of an electrical field across the plasma membrane. Initially, the efflux of K^+ will dominate, followed by greater influx driven by growing potential difference. At equilibrium, a steady state in which the tendency for further changes disappears, a membrane potential will be established based on a single ion and the selective permeability of the channels in membrane. In this hypothetical cell model, the same logic could be applied assuming the presence of a plasma membrane expressing exclusively Na^+ or Cl^- channels.

The potential difference at which the equal movements occur is called the *equilibrium potential*, also

known as *Nernst potential*. Thus, equilibrium potential is the membrane potential at which there are no net ion movements and the ion gradients and membrane potential will remain stable indefinitely.

The equilibrium potential for four major ions can be calculated using the Nernst equation:

$$E_K = RT/zF \ln[K]_o/[K]_i \quad (2)$$

$$E_{Na} = RT/zF \ln[Na]_o/[Na]_i \quad (3)$$

$$E_{Ca} = RT/zF \ln[Ca]_o/[Ca]_i \quad (4)$$

$$E_{Cl} = RT/zF \ln[Cl]_i/[Cl]_o \quad (5)$$

where R is the gas constant, T is temperature (in degrees Kelvin), z is valence of the ion, and F is the Faraday constant (the amount of electrical charge contained in one mol of a univalent ion).

1.9. Real Cells Are Not at Equilibrium

The balance of efflux and influx of K^+ in a mammalian cell-model (Table 1) would be reached at a membrane potential of -91 mV, for Na^+ the balance would be at $+67$ mV, and for Cl^- at -86 mV. The sign of the equilibrium potential reflects the polarity of the intracellular site of the cell. However, the resting potential of cells (about -60 mV) is different from the equilibrium potential for all four major ions, indicating that in the physiologic-like situation, none of them is in equilibrium and that the steady state of the resting potential is reached by other forces. The magnitudes and direction of these forces became obvious by comparing the resting potential with the equilibrium potential for a particular ion. For example, $+67$ mV equilibrium potential for Na^+ and -60 mV resting potential indicate that concentration and electrical gradient will favor entry of Na^+ , if channels are permeable for this ion. In a case of K^+ ion, the actual resting potential is not large enough to counter the force of diffusion forcing a small but constant K^+ efflux. As illustrated in Fig. 6 by length of arrows, there is more force moving Na^+ into the cells than there is moving K^+ out. At a membrane potential of -60 mV, the sum of diffusion and electrical forces (net driving force) for K^+ is 31 mV and for Na^+ it is 127 mV. However, at resting potential, only a few Na^+ -conducting channels are open. In other words, the permeability of membrane for Na^+ at resting potential is very low. There is also a small but constant K^+ efflux. Finally, the passive influx of Na^+ and efflux of K^+ is balanced by the action of the Na^+-K^+ pump, which brings two K^+ for three Na^+ that are expelled.

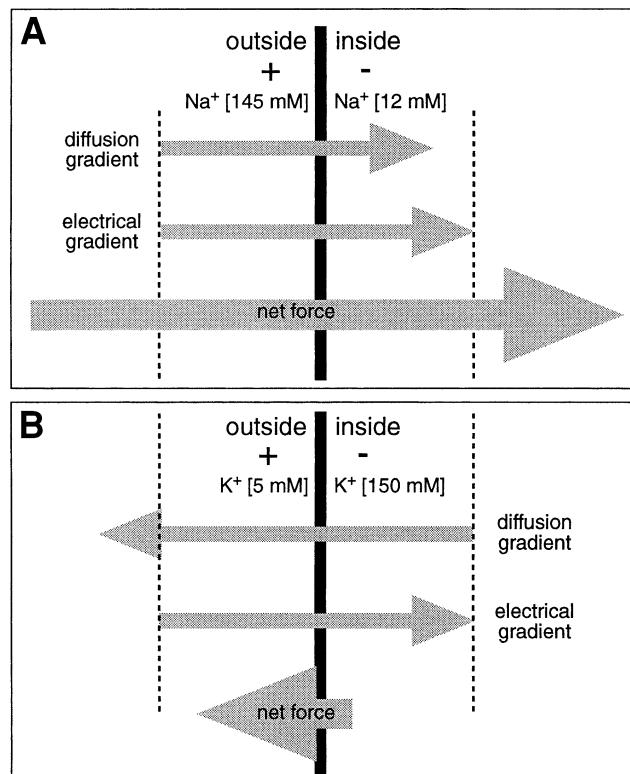


Fig. 6. Schematic representation of the magnitude and directions of passive forces acting on Na^+ (A) and K^+ (B) in a mammalian cell model. The algebraic difference between the diffusion gradient and electrical gradient indicates the direction and magnitude of the net force in a resting cell.

1.10. Multi-ion Electrochemical Equilibrium

An ion electrochemical gradient illustrates the forces on ion movement through a single channel type, but real cells express more than one ion channel type. Furthermore, the dissociation between the resting potentials and equilibrium potentials introduces a need to integrate the permeability of the membrane for ions (in addition to the concentration of the ions in two conductors), which is not a factor in the Nernst equation. Thus, in order to calculate membrane potential in a multi-ion system, we need to know the equilibrium potential for each permeant ion and the permeability for each ion. This equation was derived by Goldman (1943) and Hodgkin and Katz (1949):

$$E_m = RT/F \ln \frac{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o} \quad (6)$$

in which R , T , F , and z are as defined for the Nernst equation, and P_{Na} , P_K , and P_{Cl} are the permeabilities of the membranes to Na^+ , K^+ , and Cl^- , respectively. Because absolute permeabilities are difficult to

measure, the equation can be also expressed in terms of relative permeabilities:

$$E_m = RT/F \ln \frac{[K^+]_o + (P_{Na}/P_K)[Na^+]_o + (P_{Cl}/P_K)[Cl^-]_i}{[K^+]_i + (P_{Na}/P_K)[Na^+]_i + (P_{Cl}/P_K)[Cl^-]_o} \quad (7)$$

The frequently used permeability ratios for $P_K:P_{Na}:P_{Cl}$ are 1:0.04:0.45.

Two important conclusions are derived from ion concentrations and permeabilities. (1) The resting potential most closely approximates the calculated Nernst equilibrium potential for the most permeable ion. (2) The membrane potential is most influenced by changes in concentrations of the permeable ion. For example, if a membrane is equally permeable to Na^+ and K^+ , the membrane potential would be about -15 mV . However, because the measured resting potentials in neurons closely approximate the calculated equilibrium potential for K^+ , it is likely that the membrane is more permeable to K^+ than to Na^+ at rest.

2. ELECTRICAL EXCITABILITY OF THE CELL MEMBRANE

2.1. Excitable Cells Fire Action Potentials

Nerve cells generate electrical signals that transmit information. These signals are called *nerve impulses*, *action potentials*, or *spikes*. Action potential is a brief transient reversal of membrane potential that speeds along the axons of nerve cells but also over the membranes of many muscle and endocrine cells. It propagates regeneratively as an electrical wave without decrement and at high and constant velocity. Action potential provides an effective mechanism for rapid signaling over the long distance. The best way to observe an action potential is to use an intracellular microelectrode to record directly the electrical potential across the plasma membrane. As shown in Fig. 7, in response to an electrical shock, the membrane potential changes from its resting value toward zero; thus, a rapid *depolarization* of the membrane occurs. This is followed by *reverse polarization*, with the interior of cells being positive relative to the outside. The membrane is then seen to *repolarize*. Typically, a short *hyperpolarization* follows the initial return of membrane to resting potential. Cells that can make action potentials spontaneously or in response to an electrical shock are known as *electrically excitable*.

2.2. Neurons Also Generate a Variety of Slow Potentials

Action potential in response to an electrical shock is initiated at a particular membrane potential, called

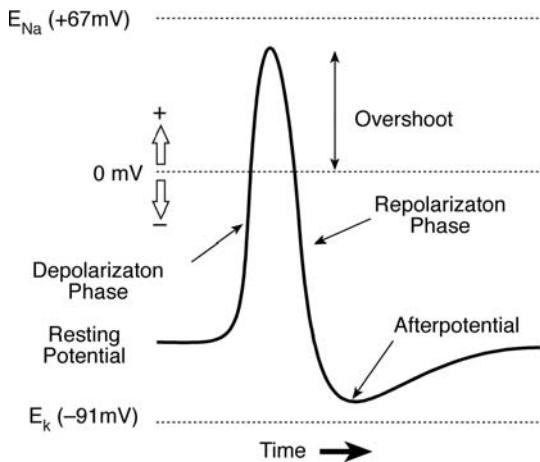


Fig. 7. Schematic representation of a neuronal action potential. The action-potential waveform is composed of several components. A rapid depolarization from a resting potential of about -60 mV leads to the reverse polarization, which peaks below the Na^+ equilibrium potential. The peak value above 0 mV is known as overshoot. The repolarization phase is usually cell type specific; in some cells it occurs rapidly, whereas in others it occurs with delays. During the repolarization phase, the membrane potential can briefly become more negative than the resting potential and is known as a hyperpolarizing afterpotential. The repolarization value usually does not exceed the K^+ equilibrium potential.

threshold. If the threshold depolarization is reached, action potential will be generated independently of further strength of the depolarizing stimulus. Also, the peak amplitude of action potential is not related to the strength of depolarizing stimulus; once it is triggered, action potential is a self-driving phenomenon. This is known as the *all-or-none* feature of action potential, in contrast with the graded nature of the voltage changes in synapses. The subthreshold stimuli produce only *localized responses*. These experimentally induced responses resemble slow potentials recorded from neurons, especially on the synaptic site of actions of neurotransmitter molecules and sensory endings. Localized responses are slow potentials that may take a depolarizing or hyperpolarizing form. At postsynaptic membranes, these responses are called *postsynaptic potentials* and in sensory endings they are called *receptor potentials* (see Chapter 5). When both postsynaptic and receptor potentials reach the threshold level, they generate action potential. Such a depolarizing “trigger” can also be induced by endogenous pacemaking activity of cells that generate action potential spontaneously. The passive flow of electrical current plays a central role not only in generation but also in propagation of action potentials. Basically, action potentials

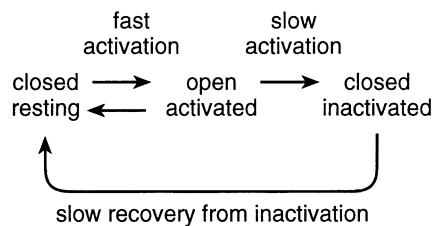
propagate along the nerve cell axons by virtue of the local current flow between the active and inactive regions of the axon.

2.3. Action Potential and Ion Movements

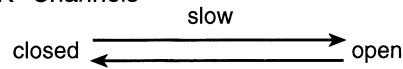
The electrochemical energy of membrane potential and the sequence of the permeability change to ions account for the generation of action potential. This was well illustrated by voltage clamp technique, an electronic method that allows control of membrane potential. At the same time, the voltage-clamp method provides the direct measurements of ion conductivity as electrical current across the cell membrane. Furthermore, by changing the solutions, it is possible to resolve the individual ionic components. In a series of elegant experiments with voltage-clamped cells, Hodgkin and Huxley were able to precisely calculate the changes in the conductance of Na^+ and K^+ during action potential. These experiments revealed that the opening and closing of Na^+ and K^+ channels is controlled by membrane potential. Hodgkin and Huxley made a kinetic model of the opening and closing for these two channels; the simplified version of this is shown in Fig. 8A. They were able to calculate the theoretical shape of the action potential from these conductance changes and to find a remarkable similarity with the recorded action potentials. Figure 8B shows the temporal relationship between channel opening and action-potential waveform.

The initiation of action potential in axons depends on Na^+ influx and in some cells on Ca^{2+} influx. Depolarization of neurons induced by an electrical shock, or generated at synapses—if sufficient strong and occurring in the region of membrane expressing $\text{Na}^+/\text{Ca}^{2+}$ and K^+ channels—triggers an increase in the probabilities of these channels to open. Na^+ channels respond more rapidly compared with K^+ channels, and the membrane permeability to Na^+ rises relative to that of K^+ . If a few Na^+ channels are open, they have a minimal effect on membrane potential. As a result, the membrane potential will return to normal. A stronger depolarization, however, will activate more Na^+ channels. Both the concentration gradient for Na^+ and the negative intracellular potential facilitate Na^+ influx, which further depolarizes the cell and activates more Na^+ channels to open and more Na^+ entry, further depolarization, and an action potential. At the peak membrane potential, the membrane is about 20 to 50 times more permeable to Na^+ than to K^+ —the reverse situation of the relative permeabilities for these two channels at resting membrane potential. Such a massive influx of positive

A Na^+ Channels



K⁺ Channels



B

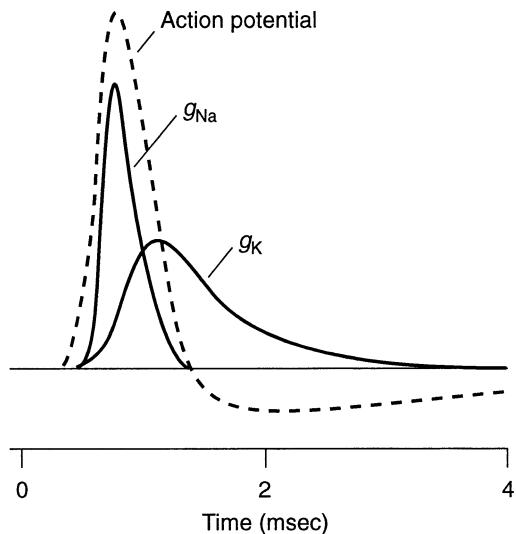


Fig. 8. Changes in conductance of Na^+ and K^+ during action potentials. (A) Simplified diagram illustrating the voltage-dependent opening and closing of Na^+ and K^+ channels. (B) Time-course of changes in membrane potential and conductance for Na^+ and K^+ channels calculated from voltage-clamped experiments. (Adapted from A.L. Hodgkin and A.F. Huxley, J Physiol 1952; 117:500.)

charge makes the inside of cells positive for a short period, and the polarity of membrane reverses.

The repolarization phase of action potential that returns membrane to the resting level results from changes in the permeability of both K^+ and Na^+ channels. The initial depolarization of cells mediated by Na^+ also increases the opening probability of K^+ channels, but slightly later than the gates of Na^+ channels. This results in a delay of the peak flow of K^+ current, which is known as delayed rectifier K^+ current. Although the membrane is depolarized, Na^+ channels became nonconducting because of a phenomenon

called *channel inactivation*. Sodium-channel inactivation kinetics are rapid. This leaves the activated K^+ channels to dominate the membrane permeability and to repolarize membrane to resting level. The continuous opening of K^+ channels at that time-point accounts for development of afterhyperpolarization. Eventually, K^+ channels close at more negative potentials, and the resting potential is restored. The peak of action potentials is always less than the equilibrium potential for Na^+ , and the hyperpolarization that follows an action potential is never more negative than equilibrium potential for K^+ . Also, the generation of typical action potentials is not affected by abolition of carrier activities, consistent with the hypothesis that changes in the permeabilities of channels and passive movement of ions are sufficient for the generation of action potential.

2.4. How Does the Action Potential Propagate?

Long-distance communication in the nervous system is possible because action potentials propagate smoothly down an axon maintaining the same amplitude. The

general principles by which action potentials propagate are similar in nonmyelinated and myelinated axons and are dependent on localized currents. The action potential-induced depolarization of cell membrane spreads a small distance in either direction inside the axon. This occurs because the intracellular and extracellular media are better conductors than is the cell membrane. As a result, an action potential-affected area smoothly depolarizes the region ahead of the action potential. Once this depolarization reaches the threshold, opening of Na^+ channels further advances the wave of excitation. Although localized current also flows into the area behind the advancing action potential, it does not develop into an action potential because Na^+ channels in this region are inactivated, and channels are in a nonconducting mode. This is known as the *refractory period*, an important feature that forces action potential to travel in only one direction (Fig. 9). The refractory period also limits the number of action potentials that neurons can produce per unit time.

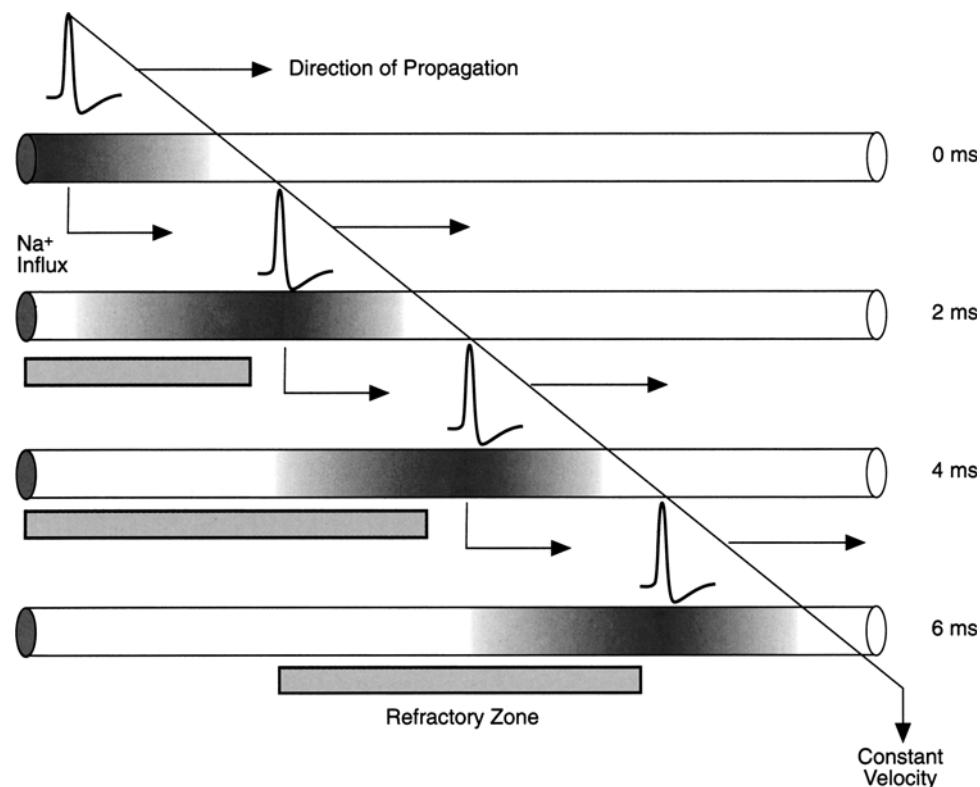


Fig. 9. The propagation of action potential along axons occurs at a constant velocity. The scheme illustrates four time-points during the propagation of an action potential. The gray areas indicate the intensity of changes in membrane potential, with the darkest area corresponding with the peak of the action potential. The localized currents flow in the front of the action potential but also into the area behind the advancing action potential. Because Na^+ channels remain in an inactivated state after action-potential depolarization, however, a refractory zone occurs, forcing action potential to travel in only one direction.

2.5. Myelin Enhances the Speed of Action Potential

The speed of action-potential propagation depends on the density of Na^+ channels and the diameter of axon. For example, the squid axons have a diameter of approximately 1 mm and conduction velocity of almost 100 m/s, whereas mammalian C-fibers have a diameter of $<2 \mu\text{m}$ and conduct with a speed of 1 to 2 m/s. Thus, the more channels are expressed per unit area and the larger diameter of axon, the more rapidly the propagation of action potential occurs. Neither of these two features is physiologically attractive, because of the energy cost and because a single nerve would be packed with only a few axons. Still, the majority of mammalian nerves are packed with hundreds of axons, with diameters of only 10 to 20 μm , but their conductance velocity is approximately 50 m/s. This has been achieved by the expression of myelin around axons, which increases the velocity of action-potential propagation.

In myelinated neurons, the majority of the axon is covered by myelin. However, the myelin wrap surrounding an individual axon is interrupted at 1- to 1.5-mm intervals, and these regions are known as *nodes of Ranvier* (about 20 μm long). A single glial cell, known as a Schwann cell, covers one *internodal area* with myelin. In such axons, depolarization also spreads from an excitable to a nonexcitable patch by localized currents, but the action potential develops only at the nodes of Ranvier (Fig. 10). The area under myelin wrap is practically nonconducting and almost entirely without Na^+ channels, and the insulating properties of myelin restrict the currents on the nodes of Ranvier. The nodal membrane expresses a high density of channel per unit compared with the nonmyelinated axons and dendrites, which helps to depolarize the long internodal myelin. The local current flow from one node to the next causes a new action potential to be generated there, skipping the myelinated area—a phenomenon known as *salutatory conduction*, which involves the jump of action potential from node to node. This in turn speeds the progression of action potential and saves on the expression of channels and carriers and energy needed for the propagation of action potentials. Not surprisingly, loss of myelin that occurs in diseases such as multiple sclerosis causes serious neurologic problems (see Clinical Correlation: Demyelinating Disorders).

2.6. Neurons Do Not Require Energy to Conduct Action Potential

The $\text{Na}^+ - \text{K}^+$ pump is needed to account for the leak of Na^+ and K^+ at rest and the small amount of

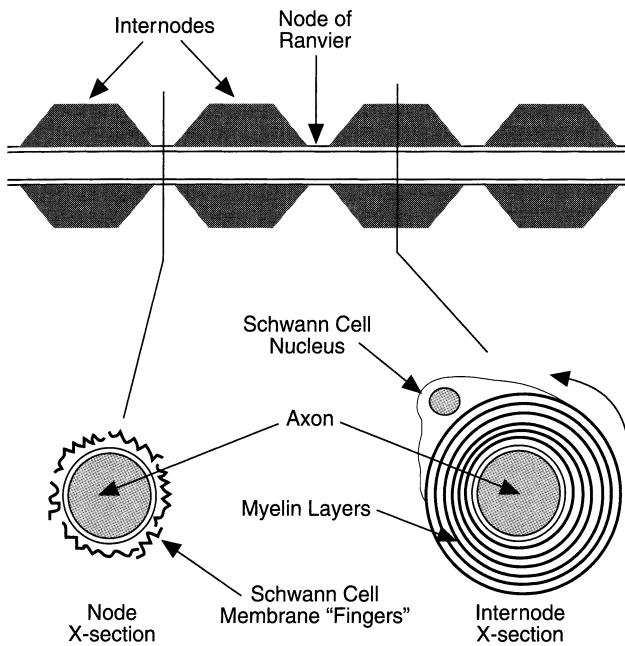


Fig. 10. Myelination of axons enhances the speed of action-potential signaling. Concentric layers of glial cell membranes known as myelin frequently envelop the nerve axons. The myelin wrap is interrupted at regular intervals, known as nodes of Ranvier. Only at these nodes is axon membrane excitable, because the myelin sheath prevents the movement of ions away from the outside of the axon. The myelinated regions between nodes are known as internodes.

these two ions transferred during the action potential. However, inhibition of ATP production does not block the action-potential firing. Such cells can conduct several action potentials without difficulty, indicating that immediate metabolic energy is not required for this process. In unmyelinated neurons, the gain of Na^+ and the lost of K^+ per action potential depend on the axon diameter. For example, in the absence of ATP, squid axon of approximately 1 mm diameter can generate about 10^5 action potentials, whereas mammalian axon of 0.2 μm diameter can only fire 10 to 15 action potentials before the intracellular Na^+ concentration would be doubled. The intracellular gain of Na^+ and the lost of K^+ per action potential in myelinated neurons are dramatically reduced because of the low-capacitance properties of myelin.

2.7. Action Potentials Can Have Different Shapes and Functions

Excitable cells differ in the pattern of action potentials and the frequency of spiking. The action potential in axons is sharp, and short in duration (*single spikes*; Fig. 11A). Many neurons, including mollusk neurons,

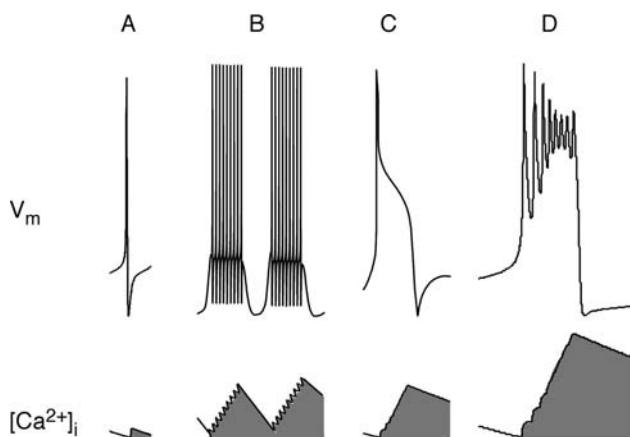


Fig. 11. Cell type-specific action-potential waveforms and calcium signals. Schematic representation of (A) a rapid single-action potential (axonal-type); (B) periodic bursting baseline firing of action potentials followed by quiescent periods; (C) plateau type of action potentials (cardiac type); and (D) plateau-bursting type of action potentials (endocrine cell type).

exhibit the *periodic bursting activity* followed by quiescent phases (Fig. 11B). Other cells, such as cardiac, fire a long-lasting *plateau-type* of action potentials (Fig. 11C), whereas pituitary somatotrophs, lactotrophs, and beta pancreatic cells generate the *plateau-bursting type* of action potentials (Fig. 11D). The pattern of action potential is determined by the types and density of channels expressed in a particular cell type. The relevance of Na^+ and K^+ channels in generating action potential in axons has been previously described. In some cells, voltage-gated Ca^{2+} channels substitute for Na^+ channels in the depolarization phase of action potential. The delayed rectifier K^+ channels and ether a-go-go (erg) channels are the key players in shaping the action potential. Other K^+ channels can influence the pattern of action potential. For example, Ca^{2+} -activated K^+ channels in interactions with delayed rectifier K^+ channels can facilitate hyperpolarization of cells and delay the spike activity for several seconds. In cells that exhibit spontaneous firing of action potentials, variable channels can play a role in pacemaking (such as T-type Ca^{2+} channels and cyclic nucleotide-regulated cation channels) or oppose pacemaking (including M and inward rectifier K^+ channels).

Action potential is a signaling element in excitable cells. Both the frequency and the shape of action potential encode the signal. In cells or in regions of cells expressing voltage-gated Ca^{2+} channels, action potentials promote Ca^{2+} influx. In such cells, the shape of action-potential waveform and the pattern

of firing determine the pattern of calcium signals. In general, rapid single-action potentials generate *localized Ca^{2+} signals*, known also as *domain Ca^{2+}* , which can be detected by nearby Ca^{2+} -activated K^+ channels, but not by fluorescent dyes. On the other hand, plateau and plateau-bursting action potentials can generate *global Ca^{2+} signals*. Periodic bursting activity followed by quiescent periods can also result in global, oscillatory Ca^{2+} signaling (Fig. 11, bottom panels).

3. VOLTAGE-GATED CHANNELS

Voltage-gated channels are the superfamily of ion channels that include sodium, calcium, and potassium channels. Several subtypes of voltage-gated chloride channels have also been identified, as well as numerous transient receptor potential (TRP) ion channels. Voltage-gated channels are macromolecular complexes in the lipid membrane containing the *aqueous pores* and *voltage-sensors*. A part of the pore known as the *ionic selectivity filter* is narrow enough to distinguish among Na^+ , K^+ , Ca^{2+} , and Cl^- . The voltage-sensor is the charged component that senses the electrical field in the membrane and drives conformation changes, leading to opening and closing of the gates near the mouth of the pore. The voltage-dependent opening of ionic channels is known as *activation*. After only a few milliseconds, or in as much as several hundred milliseconds, the channel *inactivates* and the flow of ion is again blocked. After inactivation, the channel returns to its resting state until the next membrane depolarization triggers the whole process again.

The first evidence that voltage-gated channels are discrete entities came from experiments with different drugs and toxins. Initially, tetrodotoxin (TTX) and saxitoxin were found to inhibit Na^+ current. Experiments with tetraethylammonium ion in the presence and absence of TTX helped to identify K^+ channels. With time, the list of useful blockers of K^+ channels increased progressively and included Cs^+ , Ba^{2+} , 4-aminopyridine, apamin, and charybdotoxin. The identification and development of drugs and toxins useful for the characterization of voltage-gated Ca^{2+} channels also progressed, and included dihydropyridines, verapamil, Cd^{2+} , Ni^{2+} , and ω -conotoxin GVIA. A part of ion channel molecule operates as receptor, by binding a drug in a specific and reversible manner. In many cases, this has been used to quantify channels in specific tissues. Binding of drugs also affects the conductivity of channels. Two sister compounds may have opposite effects on conductivity.

For example, nifedipine acts as an antagonist and BayK 8644 acts as an agonist for L-type voltage-gated Ca^{2+} channels.

Combined pharmacologic and electrophysiologic experiments have revealed that there is a high diversity of K^+ and TRP channels, whereas voltage-gated Ca^{2+} and Na^+ channels are less diverse. The functional properties of Na^+ channels are relative similar, in contrast with K^+ , Ca^{2+} , and TRP channels. The same combination of tools, as well as the use of fluorescent antibodies for specific ion channels, has also confirmed that ion channels can be highly localized. In addition to the nodes of Ranvier, these include dendrites, synaptic boutons, and nerve terminals. Finally, recent molecular biology- and protein chemistry-based techniques have provided a great deal of information about the structure of this superfamily of more than 140 members, representing one of the largest groups of signal transduction proteins. Voltage-gated channels are composed of the pore-forming subunits and auxiliary subunits. A remarkable finding was that the pore-forming subunit of Na^+ channels and of Ca^{2+} channels have similar amino acid sequences and folding. The pore-forming subunit of K^+ channels is smaller, but with obvious homology to Na^+ and Ca^{2+} channels. In rodents, 10 genes for the Na^+ -channel family, 10 genes for the

Ca^{2+} -channel family, and more than 100 genes for the K^+ -channel family have been identified.

3.1. Gigaseal and Patch-Clamp Methods

Earlier studies by Hodgkin, Huxley, and Katz suggested the presence of discrete ion-channel proteins with an aqueous pore permeable to ions. Neher and Sakmann confirmed this hypothesis. They developed the *patch-clamp* method for single-channel recording, which uses glass electrodes with the tip opening of several micrometers in diameter and with a smooth surface achieved by heat polishing. By pressing the pipette against the living cells, they recorded a single-channel current with an acetylcholine-activated channel for the first time. Hamill and collaborators further developed this technique by showing that the pipette can fuse with membrane to form a high-resistance seal. As shown in Fig. 12, this can be achieved by application of gentle suction, drawing a small patch of membrane into the electrode opening. After a few seconds, an unexpectedly high resistance and mechanical stability is achieved between the membrane and the glass surface, with a negligible flow of ions between the two surfaces. This seal is known as a *gigaseal* because electrical resistances between the inside of the electrode and the extracellular fluid are in tens of gigaohms. If the patch of membrane

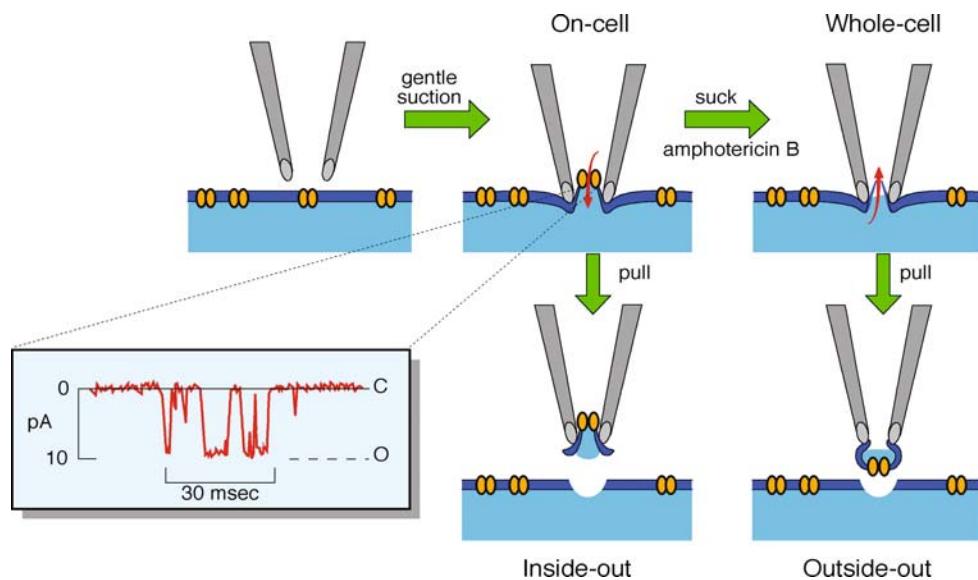


Fig. 12. The patch-clamp recording methods. All patch-clamp methods use glass electrodes with a tip opening of several micrometers in diameter and with a smooth polished surface. The methods start with placing the electrode against an intact cell and application of gentle suction. After establishing a strong bond, the voltage of the electrode interior can be clamped at any level, and single channels can be recorded in this on-cell mode. The inset illustrates sporadic opening of Ca^{2+} -activated K^+ channels (o, open, conducting state; c, closed state). As indicated in the figure, the same electrode can be used to obtain the addition patch-clamp configurations.

contains an individual ion channel, most of the current passes from the electrode flow through the single channel. This model of recording is known as *on-cell* or *cell-attached patch* mode, and the inset of Fig. 12 illustrates K⁺-current movement through Ca²⁺-controlled K⁺ channels recorded by this method.

The gigaseal permits three additional modes of recording. The seal between the membrane and pipette is so tight that its withdrawal frequently rips the patch of membrane from the cell. The patch is sealed to the pipette and can be bathed in variety of solutions. This configuration is termed *inside-out* or *excised-patch* mode (Fig. 12). The cell-attached patch can be ruptured by suction without affecting the seal to the cell, or the permeabilization of the patch membrane can be achieved by antibiotics, including nystatin and amphotericin. These configurations are known as *whole-cell* and *perforated-cell* modes, respectively. In the first model, the interior of cells is dialyzed in a short time by the recording pipette solution. The perforated patch membrane is semipermeable; it is usually permeable only for monovalent ions. Finally, from the whole-cell mode, one can achieve the *outside-out* patch mode by pulling the pipette away from the cell (Fig. 12).

3.2. Voltage-Gated Na⁺ Channels Depolarize Cells

Voltage-gated Na⁺ (Na_v) channels consist of the pore-forming α subunit associated with auxiliary β subunits. The α subunit is sufficient for functional expression, but the channel gating is modulated by the β subunits. Mammals express nine genes for α subunit (Na_v1.1 to Na_v1.9), and the dendrogram shown in Fig. 13 indicates the similarity in their structures. Closely related Na⁺ channel-like proteins (Na_x) have also been cloned from several mammalian species, with approximately 50% similarity in the structure with Na_v1 channels. The α subunits of Na_v channels can be classified into two general groups: TTX-sensitive and TTX-insensitive (Fig. 13). To date, four auxiliary subunits have been identified and termed Nav β ₁, Nav β ₂, Nav β ₃, and Nav β ₄. They belong to a single family of proteins, which interacts with different α subunits and alter their physiologic properties.

The structural transmembrane folding model of Na_v channels is shown in Fig. 14. The four repeated domains of α subunit have greater than 50% internal sequence identity. Each domain contains six segments that make TM- α helices, whereas β subunits have a single TM domain, a large N-terminal

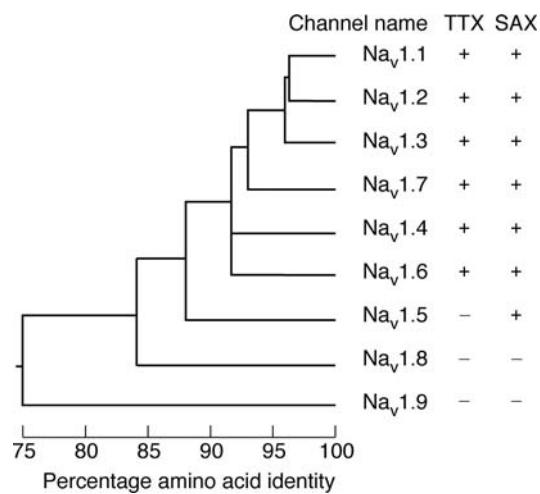


Fig. 13. Amino acid sequence similarity of voltage-gated Na⁺ (Na_v) channels. Tetrodotoxin (TTX) and saxitoxin (SAX) are sodium channel blockers. In addition to Na_v1.1-Na_v1.9 subunits, closely related sodium channel-like proteins have been cloned from several mammals and termed Na_x. (Derived from B. Hille, 2001, Sinauer Associates, Inc.; and W.A. Catterall et al., Pharmacol Rev 2005; 57:397–409.)

ectodomain, and a short C-terminal intracellular domain. When expressed, the α subunit of skeletal muscle accounts for TTX binding site, pore, voltage gate and sensor, and contains the sites for phosphorylation by protein kinases on the intracellular surface. The direct evidence in favor of the hypothesis that S4 segment serves as a voltage sensor comes from mutagenesis studies with Na⁺ and K⁺ channels. These segments are highly conserved among voltage-gated channels and consist of repeated triplets of two hydrophobic residues followed by a positively charged amino acid. Neutralization of positive charges leads to progressive reduction of the steepness of voltage-dependent gating, as expected for a voltage sensor. However, a single cluster of three hydrophobic amino acids in the intracellular loop connecting 3TM and 4TM domain is required for inactivation of Na⁺ channels. The substitution of these residues with hydrophilic ones leads to generation of a noninactivating channel. It appears that Phe¹⁴⁸⁹ is a critical residue. A “hinged-lead model” was proposed to explain the mechanism of inactivation of this channel. According to this model, a cluster of hydrophobic residues together with Phe¹⁴⁸⁹ enters the intracellular mouth of the pore, providing an effective latch to keep the channel inactivated in depolarized cells.

The sensitivity of native channels to TTX varies, depending on tissue; nerve and skeletal-muscle Na⁺ channels are more sensitive to TTX than is the

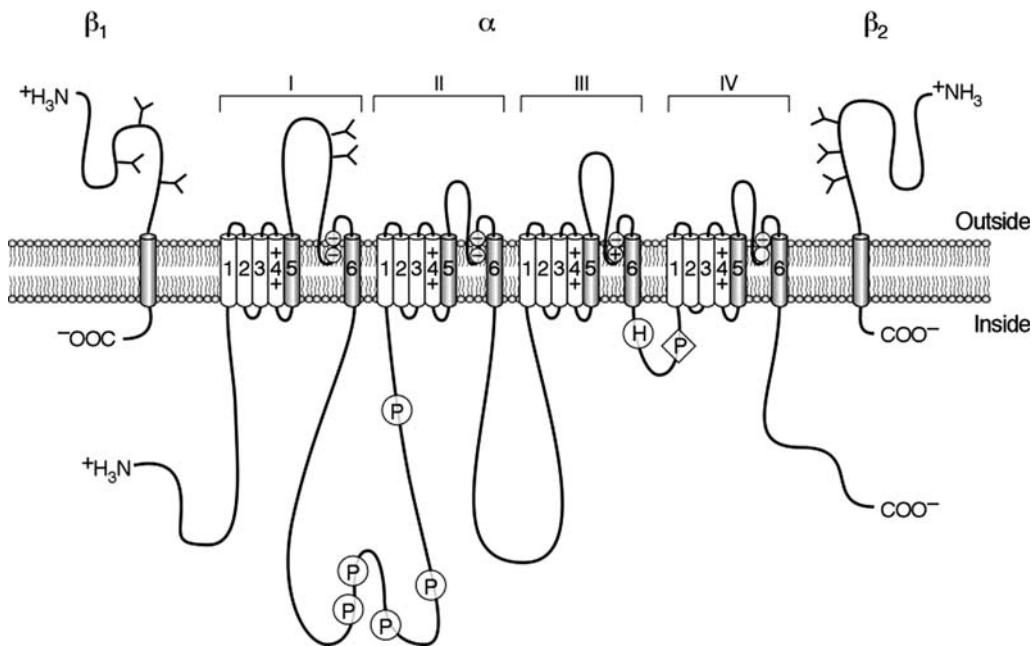


Fig. 14. Structural transmembrane folding model of voltage-gated Na^+ channels. In this and following figures, α -helices are illustrated as cylinders, and extracellular and intracellular chains of amino acids as full lines. Positively charged 4TM domain illustrates voltage-sensor. P illustrates sites for phosphorylation by protein kinases A and C.

cardiac channel. Mammalian TTX-sensitive Na_v channels expressed in the brain are composed of large (260,000) α subunit associated with β_1 (36,000) and β_2 (32,000) polypeptides, whereas Na_v channels in skeletal muscle are composed only of α and β_1 subunit. As discussed earlier, the main function of these channels is to depolarize cells and generate the upstroke of the action potential and to control the firing amplitude in excitable cells, including nerve, muscle, and neuroendocrine cell types. In some cells, these channels are solely responsible for the rapid and regenerative upstroke of an action potential. In others, they act in conjunction with voltage-gated Ca^{2+} channels to depolarize cells. The channels from this family can also control pacemaking. Na_v channels are also expressed in nonexcitable cells at lower level, where their physiologic role is unclear. There are significant kinetic differences between the fast TTX-sensitive and the slower TTX-insensitive Na_v channels, as well as the differences in the pattern of action-potential waveforms in cells expressing these channels. TTX-sensitive Na_v channels inactivate almost completely with depolarization to 0 mV and beyond. A subtype of these channels, however, does not show complete inactivation. TTX-sensitive channels are permeable to Na^+ , and also for K^+ , but less well (7% to 10% of that for Na^+), and to several other ions with order $\text{Na}^+ = \text{Li}^+ > \text{Tl}^+ > \text{K}^+ > \text{Rb}^+ > \text{Cs}^+$.

3.3. Voltage-Gated Calcium Channels Have Dual Functions

The Ca^{2+} -selectivity and voltage-sensitivity of these channels are common features between two major groups of *voltage-gated Ca^{2+} (Ca_v) channels*, which are separated by their sensitivity to changes in membrane potential. The first group of channels requires only weak membrane depolarization to open. Consequently, they are activated at relatively hyperpolarized membrane potentials and are known as *low-voltage activated (LVA) Ca_v* channels. Activation of these channels is followed by their rapid and complete inactivation, and a strong membrane hyperpolarization is required to bring them out of steady inactivation. Because of such inactivation properties, these channels are often referred to as transient or T-type Ca_v channels. The second group of Ca_v channels requires moderate to strong membrane depolarization to open and are known as *high-voltage activated (HVA) Ca_v* channels. Among this group, biophysical and pharmacologic studies have identified multiple subtypes that can be distinguished by their single-channel conductance, pharmacology, and metabolic regulation: L-, N-, P/Q-, and R-type Ca^{2+} channels. The L-type Ca_v channels are sensitive to dihydropyridines and exhibit slow inactivation. N-, P/Q-, and R-type channels inactivate rapidly but incompletely and are dihydropyridine-insensitive. N-type channels are sensitive to

ω -conotoxin GVIA, P/Q channels are sensitive to w-Agatoxin-IVA, and R-type channels are resistant to both toxins. T-type channels are sensitive to nickel in low micromolar concentrations.

The first Ca^{2+} channel was purified from skeletal muscle, as it is a highly enriched source of L-type channels. Purification of the channel has identified five subunits, a large α_1 (200 to 260 kDa) subunit and four smaller ancillary subunits: α_2 , β , γ , and δ . Since then, several other isoforms have been identified (Fig. 15). Consistent with the functional studies, molecular cloning and sequence comparison indicates the presence of three subfamilies of these channels: L-, N-, and T-like channels. The L-group is composed of at least four genes ($\text{Ca}_v1.1$ to 1.4), N-group is composed of three genes ($\text{Ca}_v2.1$ to 2.3), as well as the T-group of channels ($\text{Ca}_v3.1$ to 3.3). The complexity of these channels is further increased by alternative splicing. In general, Ca_v channels have diverged much more from each other than Na^+ channels. The α_1 subunit consists of four homologous repeats, each one composed of six TM segments (Fig. 16). Located within the α_1 subunit are the voltage sensor, gating machinery, channel pore, and most of the known sites of channel regulation by intracellular messengers, drugs, and toxins, including multiple PKA phosphorylation sites. The S4 segment serves as the voltage sensor, whereas the pore loop between TM5 and TM6 segments in each domain determines ion conductance and selectivity.

Coexpression of several Ca^{2+} -channel subtypes in a single cell is common in neurons and neuroendocrine cells. For example, both T- and L-type Ca^{2+} channels are expressed in excitable endocrine cells. In sensory neurons, T-type and L-type Ca^{2+} channels are coexpressed with N-type Ca^{2+} channels. In other neurons, P/Q-type Ca_v channels are also found in conjunction with other subtypes. Although multiple Ca^{2+} -channel subtypes may be coexpressed in the same cell, they are often distributed nonuniformly in different regions. In inferior olfactory neurons, HVA Ca_v channels are found mostly, but not exclusively, in the dendrites, whereas LVA Ca_v channels are usually found in the cell body. The distribution of HVA Ca_v -channel subtypes within the same cell may also be nonuniform. In neurons, extensive expression of the α_1 subunits of the N- and P/Q-type Ca_v channels has been found in the dendritic shafts and presynaptic nerve terminals, but not the cell body. Conversely, the α_1 subunits of L-type Ca_v channels were found predominately in the soma and proximal dendrites, whereas the α_1 subunit of the R-type Ca^{2+} channel

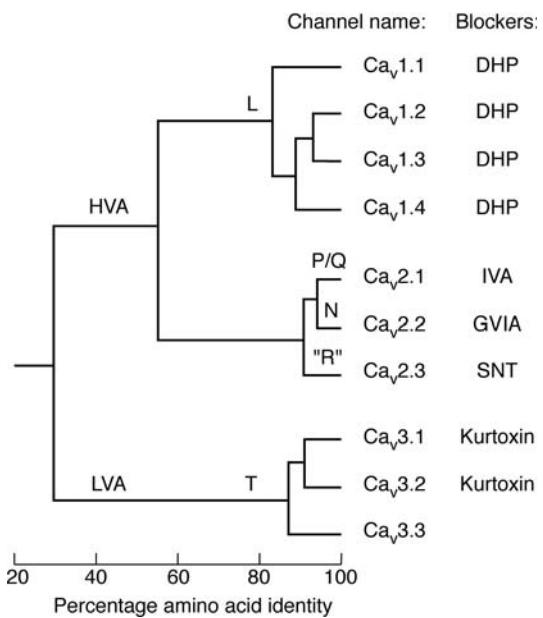


Fig. 15. Classification of voltage-gated Ca^{2+} (Ca_v) channel α_1 subunits. VGCCs, voltage-gated Ca^{2+} channels; HVA, high voltage activated; LVA, low voltage activated; DHP, dihydropyridines; w-Agatoxin (IVA); w-Conotoxin (GVIA); SNK-482 (SNT). (Derived from B. Hille, 2001, Sinauer Associates, Inc.; and W.A. Catterall et al., Pharmacol Rev 2005; 57:411–425.)

was found predominately in the cell body of central nervous system (CNS) neurons. The nonuniform distribution of Ca^{2+} -channel subtypes likely reflects their different functional roles.

Ca_v channels serve two major functions in cells: electrogenic and regulatory. In some neurons and many neuroendocrine cells, these channels give rise to action potentials in the same way as Na_v channels. In other neurons, voltage-gated Ca_v channels shape the Na^+ -dependent action potentials. The regulatory function of these channels is based on Ca^{2+} influx during the transient depolarization, which acts as an intracellular (second) messenger controlling a variety of cellular functions, and this function is comparable with the actions of membrane receptors. The most important process regulated by these channels is the release of neurotransmitters at synapses. Ca_v channels differ in their activation and inactivation properties, which makes them preferential for electrogenic or regulatory processes. LVA Ca_v channels exhibit rapid and complete voltage-dependent inactivation and are unlikely candidates to promote sufficient Ca^{2+} influx. The major function of these channels is electrogenic; at the resting potential, T-type channels depolarize cells to the threshold level for Na^+ or Ca^{2+} spike. In contrast, HVA channels inactivate incompletely and are solely responsible to keeping

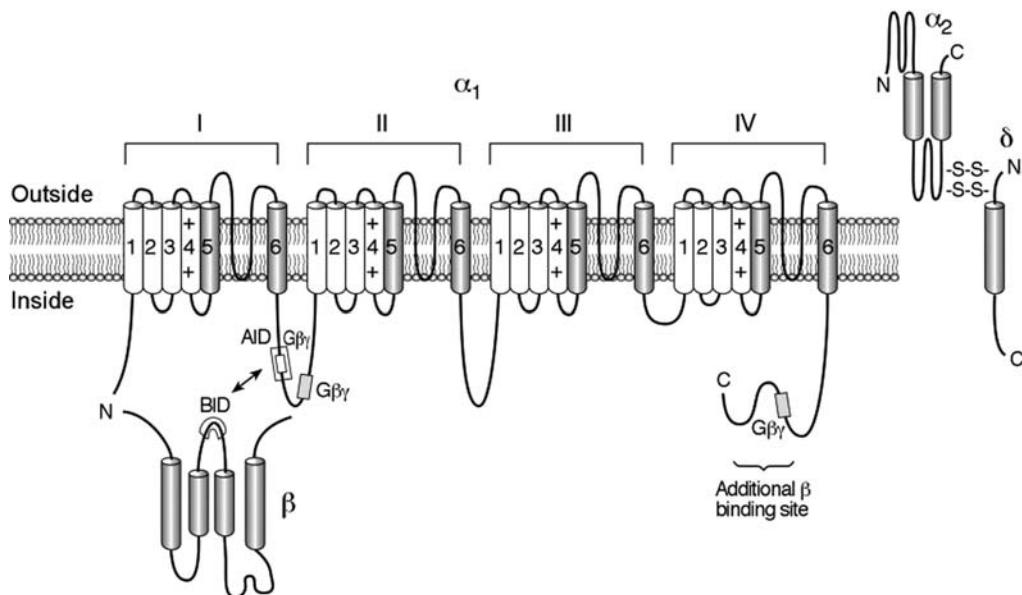


Fig. 16. Structural transmembrane folding model of voltage-gated Ca^{2+} channel complex. Diagram indicates the putative transmembrane topologies of α_1 subunit, as well as α_2 , β , and δ subunits. The binding sites for $\text{G}\beta\gamma$ dimer are also shown. (Derived from A.C. Dolphin, J Physiol 1998; 506:3.).

the cells depolarized for a prolonged period. Such action potentials drive Ca^{2+} to generate intracellular Ca^{2+} signals of sufficient amplitude to trigger Ca^{2+} -dependent processes such as neurotransmission. In accordance with this, HVA channels are found at synaptic endings and Na^+ channels in axons.

3.4. Potassium Channels Tend to Reduce Excitation

All potassium channels have a similar permeability mechanism. They contain the K^+ -channel signature sequence in the selectivity filter and show comparable ion selectivity order ($\text{Tl}^+ > \text{K}^+ > \text{Rb}^+ > \text{NH}_4^+$). Their permeability for Na^+ and Li^+ is low. These channels are usually blocked by Cs^+ , as well as by tetraethylammonium from the inside and outside, and some of them by Ba^{2+} . Their opening is frequently modulated by receptors—through G-protein action directly as well as indirectly—through intracellular messengers and kinases, including Ca^{2+} , cyclic nucleotides, ATP, PKA, PKC, and PKG.

Molecular biology-based experiments revealed an impressive structural and functional diversity of K^+ channels (Fig. 17). The principal element of all K^+ channels is the two-TM segment, which is analogous in structure and function to the S5 and S6 segments of Na_v and Ca_v channels. *Inwardly rectifying K⁺* (K_{ir}) channels and bacterial K^+ channel known as KcsA represents tetramers (complexes of four subunits)

that each have only these two TM segments. In the *two pore motif K⁺* ($\text{K}_{2\text{P}}$) channels, these two pore motifs are linked together (Fig. 17). *Voltage-gated K⁺* (K_v) channels are composed of tetramers of α subunits that contains these two TM domains plus additional four plasma membrane domains, together resembling one 6TM segments of Na_v and Ca_v channels. *Calcium-activated K⁺* channels (K_{Ca}), *cyclic nucleotide-gated (CNG) channels*, *hyperpolarization-activated cyclic nucleotide-modulated (HCN) channels*, and *transient receptor-potential (TRP) channels* also have this type of architecture (Fig. 17). Both 2TM and 6TM channels are homo- or heterotetramers of principal subunits, frequently associated with auxiliary β subunits.

Figure 18 shows the phylogenetic tree reconstruction for K_v1 to 9 and K_v10 to 12 families. This analysis was based on amino acid sequence alignment of the entire hydrophobic core of the channels. Several members of these channels have coding regions made up of several exons that are alternatively spliced, which further increases variations in the structures of the pore-forming subunits. However, the complexity of native channels does not arise only from distinct genes that encode them but also from their organization in tetramers. Some of K_v channels are homotetramers, but the majority of them are heterotetramers formed between different subunits within the same family. The K_v5 , 6, 8, and 9 encode subunits that do

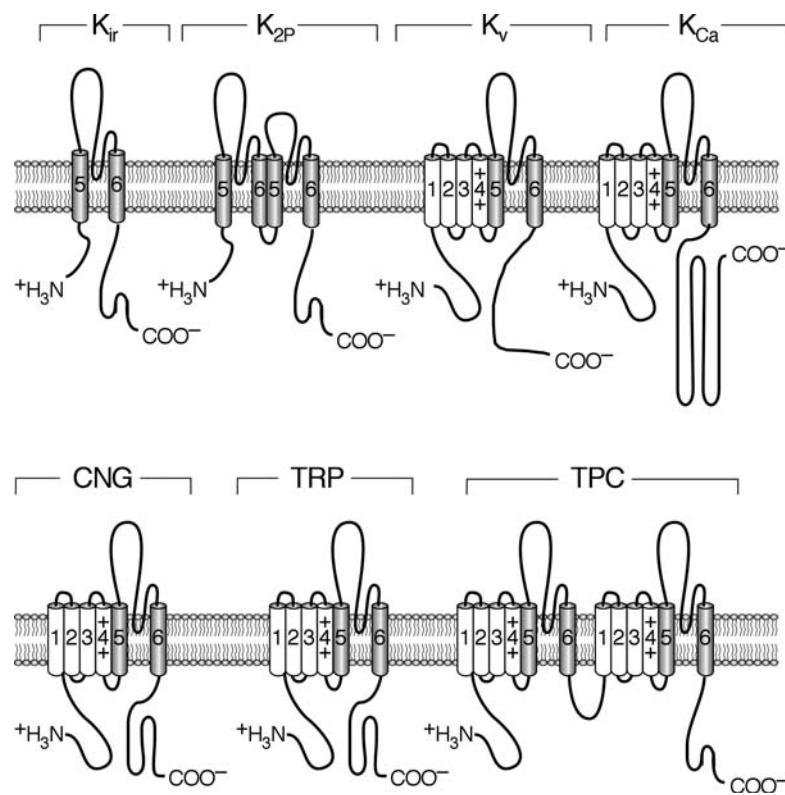


Fig. 17. Structural transmembrane folding model of K^+ and related channels. K_{ir} , inward rectifier K^+ channel; K_{2P} , two-P K^+ channel; K_v , voltage-gated K^+ channel; K_{Ca} , Ca^{2+} -activated K^+ channel; CNG, cyclic nucleotide-gated channel; TRP, transient receptor potential channel; TPC, two-pore channel.

not produce functional channels when expressed as homomers but act as modifier when expressed as heteromers, further increasing the functional diversity within this family. Assembly of these channels with accessory proteins, such as β subunits and calmodulin, and posttranslational modification such as phosphorylation further contribute to the variations between native channels.

Potassium channels are easily distinguishable by their gating characteristics. There are two classes of *delayed rectifiers*, fast and slow, which serve different functions in neurons. Fast (also known as *rapidly activated*) *delayed rectifier* belongs to the K_v class of channels. This channel is expressed in unmyelinated axons, motoneurons, and fast skeletal muscle and is responsible for very short action potentials. *Slow delayed rectifiers* expressed in cardiac tissue are from erg and KCNQ classes (K_v7) and are also involved in repolarization of cells. As indicated by their name, the gating kinetics of these channels is slow, which is reflected on the shape of the action potential. Erg channels are also expressed in pituitary cells and play a role in control of spontaneous and agonist-induced electrical activity. Neuronal and

endocrine slow-gating KCNQ channels cannot be activated by a single action potential because they gate too slowly but play an important role in control of resting potential. These channels do not inactivate and are partially open at the resting potential. The best-known member of these channels is the *M-channel*, which is made up of several subunits from the KCNQ family. The voltage-dependent activity of this channel is modulated by phosphatidylinositol 4,5-bisphosphate. A single cell frequently expresses several types of delayed rectifiers.

Another group of K_v channels is known by several names: *fast transient*, *transient outward*, *rapidly inactivating*, and *A-channels*. These channels are activated when cells are depolarized after prolonged hyperpolarization. A heterogeneous variety of gene products from the K_v family accounts for their formation. In steady state, this channel conducts in narrow (-65 to -40 mV) negative voltage range. Because of their rapid inactivation, these channels play important roles in repetitive firing by opposing the developing interspike depolarization. A single action potential is sufficient to inactivate these channels, and repolarization/hyperpolarization of membrane results from the

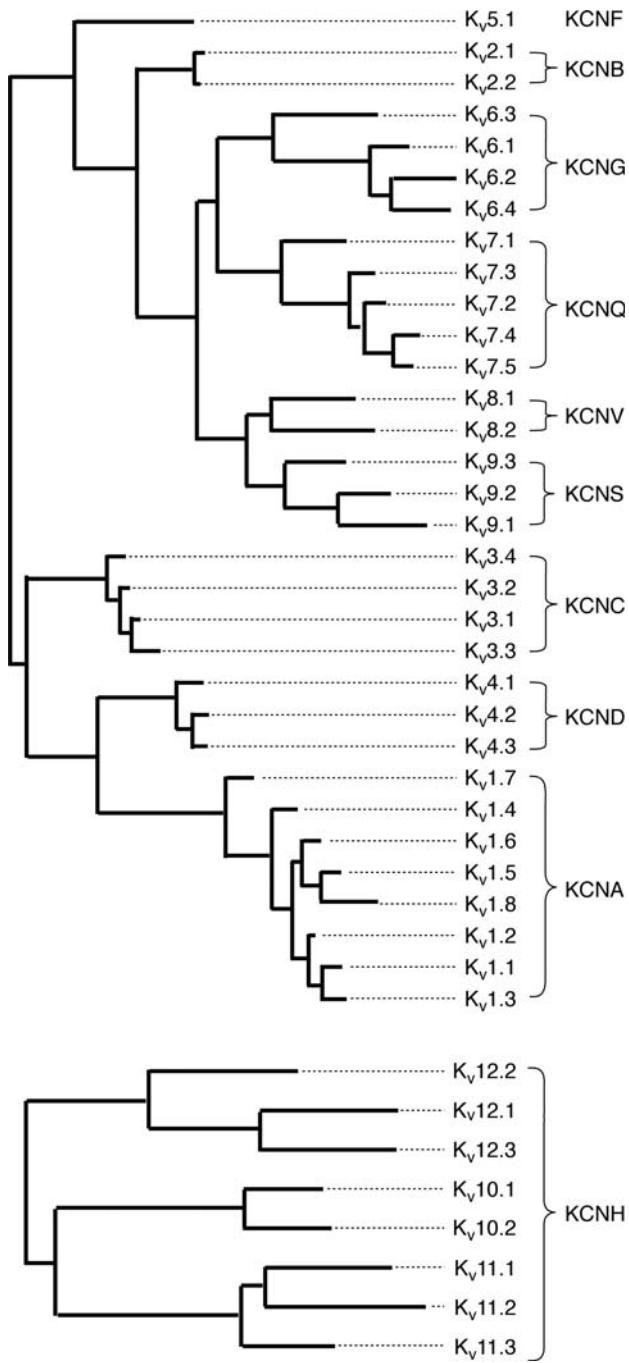


Fig. 18. Phylogenetic trees for the human $K_v 1$ to 9 and $K_v 1$ to 12 families of voltage-gated K^+ channels. The International Union of Pharmacology names are listed on the left, and the corresponding gene names established by the HUGO Gene Nomenclature Committee (HCNC) are listed on the right (see <http://www.genenames.org>). (Derived from G.A. Gutman, K.G. Chandy, S. Grissmer, M. Lazdunski, D. McKinnon, L.A. Pardo, G.A. Robertson, B. Rudy, M.C. Sanguinetti, W. Stuhmer, and X. Wang., Pharmacol Rev 2005; 57:473.)

activity of other K^+ channels. During a hyperpolarizing period, A-channels recover from inactivation, whereas other channels are shut off. This allows activation of depolarizing currents, which are for some time controlled by A-channels. However, because of their progressive depolarization, a balance is lost with time, and cells fire another action potential. Thus, in such neurons A-channels control the frequency of firing.

A-channels are the first K^+ channels to be cloned and the first to be mutated in order to clarify the gating mechanism. The N-terminus of these channels serves as an inactive particle, and all four N-termini are involved in this process known as “a ball-and-chain” mechanism. According to this model of inactivation, the N-terminal segment serves as a tethered ball that binds to the intracellular mouth of the pore. In an intact channel, any of four balls can do it. The mutant channel with deleted N-terminus does not inactivate, whereas inactivation is restored by injection of synthetic peptides with the identical amino acid sequence as native N-termini. In contrast with Na_v channels, where the critical residue for inactivation is located just 12 amino acids away from the membrane, the inactivation particle of A-channel is located more than 200 residues from the plasma membrane.

The second major group of 6TM K^+ channels consists of the *calcium-activated K^+ (K_{Ca}) channels*. The phylogenetic tree shown in Fig. 19 indicates that

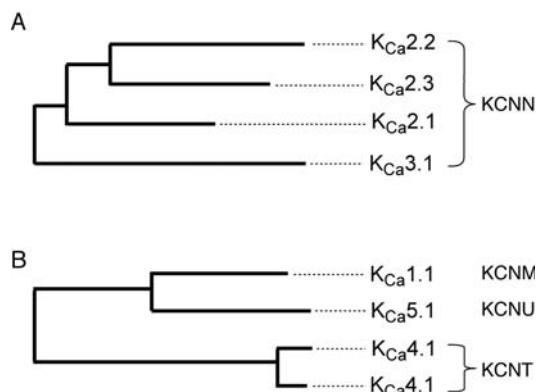


Fig. 19. Phylogenetic tree for K_{Ca} channels. **(A)** $K_{Ca} 2.1$, 2.2, and 2.3 form voltage-insensitive “small-conductance K_{Ca} channels” (SK), sensitive to block by apamin, whereas $K_{Ca} 3.1$ forms “intermediate conductance K^+ channels” (IK). **(B)** $K_{Ca} 1.1$ forms “big-conductance K^+ channels” (BK). $K_{Ca} 4.1$, 4.2, and 5.2 are a structurally related group of channels that are not activated by Ca^{2+} . The corresponding HCNC names are shown on right. (Derived from A.D. Wei, G.A. Gutman, R. Aldrich, G. Chandy, S. Grissmer, and H. Wulff, Pharmacol Rev 2005; 57:463.)

these channels belong to two families. The first group includes the so-called small-conductance (SK) K_{Ca} channels as well as the intermediate-conductance (IK) channels. These channels have little voltage-dependence and are predominately activated by calcium released from intracellular stores. Both channels are only distantly related to big-conductance (BK) channels, which are formed by $K_{Ca}1.1$ and are activated by both voltage-gated Ca^{2+} influx and release of Ca^{2+} from intracellular stores. SK channels are sensitive to block by apamin. BK channels may be blocked by charybdotoxin, iberiotoxin, and paxillin. There are at least three reasons why K_{Ca} channels are incorporated into the Ca^{2+} signaling pathway. First, activation of these channels may relieve the steady inactivation of Na_v and Ca_v channels, which stimulates or enhances action potential generation in some cells. Second, activation of K_{Ca} channels may prevent a lethal increase in intracellular Ca^{2+} concentration by limiting voltage-gated Ca^{2+} influx. Finally, activation of K_{Ca} channels and the resulting membrane hyperpolarization may serve to synchronize electrical activity and secretion in cell networks.

The third major group of K^+ channels consists of seven subfamilies of *inwardly rectifying K^+* (K_{ir}) channels (Fig. 20). These channels are also known as *anomalous rectifier*, because their conductance increases under hyperpolarization and decreases

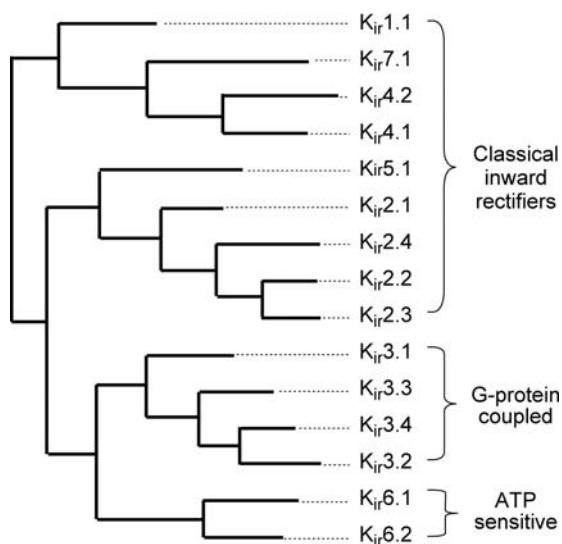


Fig. 20. Phylogenetic tree of K_{ir} channels. The 15 known members of this family of channels are divided into three groups, based on their regulation. (Derived from Y. Kubo, J.P. Adelman, D.E. Clapham, L.Y. Jan, A. Karschin, Y. Kurachi, M. Lazdunski, C.G. Nichols, S. Seino, and A. Vandenberg, Pharmacol Rev 2005; 57:509.)

under depolarization. The term *inward rectifier* describes their activation of inward current under hyperpolarization, leading to K^+ influx, and almost no K^+ efflux under depolarization. However, this small K^+ efflux carries their usual physiologic function. K_{ir} channels are expressed in numerous tissues, including brain, heart, kidney, endocrine cells, ears, and retina. These channels participate in the control of resting potential, and a strong depolarization closes them. The majority of channels are controlled by intracellular messengers. For example, the members of $K_{ir}1$ and $K_{ir}2$ are regulated by PKA and PKC, $K_{ir}3$ by G proteins, and $K_{ir}6$ by intracellular ATP. A long cytoplasmic pore of these channels plays a critical role for inward rectification and provides the structure basis for modulation of gating by G proteins and phosphatidylinositol 4,5-bisphosphate.

The existence of leak K^+ currents, which are present in resting cells and stabilize membrane potential below firing threshold, was known for more than 40 years before the structure of the underlying channels was clarified. These channels have two P loops and four TM domains, a topology similar to a tandem fusion of two K_{ir} channels (Fig. 17). The pore selectivity sequence of the K_{2P} channels is similar to other K^+ channels. Figure 21 illustrates the phylogenetic tree for 15 known K_{2P} channels, which belong to one (KCNG) family of genes. Low sequence homology

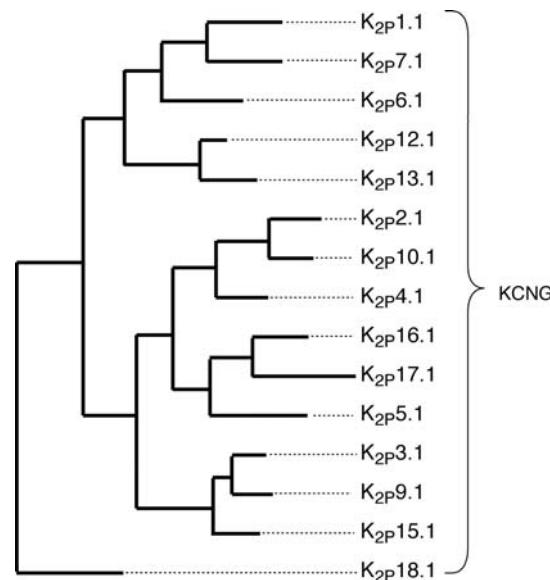


Fig. 21. Phylogenetic tree of K_{2P} channels. These channels are generated by the subfamily G of K_v channels (KCNG) family of genes. (Derived from S.A. Goldstein, D.A. Bayliss, D. Kim, F. Lesage, L.D. Plant, and S. Rajan, Pharmacol Rev 2005; 57:527.)

among K₂P channels suggests that each gene constitutes a distinct subfamily. These channels are expressed in both excitable and nonexcitable cells and conduct background K⁺ current. They are resistant to most K⁺ channel blockers, but their activity is enhanced by inhalation anesthetics, leading to hyperpolarization of neurons and reduced excitability.

3.5. Cyclic Nucleotide–Modulated Channels Are Nonselective Cation Channels

The superfamily of K⁺ channels has two members, *hyperpolarization-activated cyclic nucleotide-gated* (HCN; known also as I_h, where h stands for hyperpolarization) *channels* and *cyclic nucleotide-gated* (CNG) *channels*, which share the structure with K_V and K_{Ca} channels (i.e., they contain six TM helices and assemble in tetramers). However, HCN and CNG channels functionally dissociate from other 6TM domain K⁺ channels. Their activation does not damp excitation, but it increases the firing of action potential. Such a paradoxical role for channels that structurally belong to the K⁺-channel family comes from their permeability properties; both channels are cation-nonsselective. These channels are activated by cyclic nucleotides. The intracellular actions of cAMP and cGMP are usually mediated by their protein kinases, but HCN and CNG channels are directly activated by cyclic nucleotides.

In mammals, the HCN channel family comprises four members (HCN1 to 4; Fig. 22). When expressed alone, all four subunits form functional channels, but the native channels are probably organized as heterotetramers. Like K_{ir} channels, HCN channels are

activated by hyperpolarization beyond -60 mV, do not inactivate, and conduct Na^+ and K^+ . In cells expressing these channels, their activation leads to slow depolarization, an action consistent with their equilibrium potential of about -30 mV. HCN channels were first identified in cardiac sinoatrial node cells and subsequently in a variety of peripheral and central neurons. Their voltage sensitivity is modulated by cAMP. HCN channels serve three principal functions in excitable cells: (1) they determine the resting potential in cells; (2) they generate or contribute to the pacemaker depolarization that controls rhythmic activity in spontaneously firing cells; and (3) they compensate for inhibitory postsynaptic potentials. A small fraction of HCN channels is tonically activated at rest and determines the first two functions of these channels. In accordance with this, inhibition of spontaneously active HCN channels by low extracellular Cs^+ results in hyperpolarization of cells and abolition of spontaneous firing. Also, hyperpolarization of cells by inhibitory postsynaptic potential will increase HCN current. Furthermore, in sinoatrial node cells, the hyperpolarization that follows action potential activates these channels, leading to slow depolarization toward the threshold for new action potential. β -Adrenergic receptor-mediated stimulation of cAMP production enhances the size and speed of HCN current, resulting in an increase in the firing frequency and heart rate. In thalamic neurons, these channels are crucial in generating the rhythmic bursts of action potentials.

CNG channels are expressed in olfactory neurons and outer segments of rod and cone photoreceptors, where they play a critical role in sensory transduction. A low level of mRNA transcripts for these channels is also found in brain, pituitary, testes, kidney, and heart. Molecular cloning has revealed more than 20 genes that encode different subtypes of CNG- α subunits in invertebrates and vertebrates. These channels are heterotetramers composed of homologous A subunits (CNGA1 to A4; Fig. 22). Rod channels contain 3 CNGA1 and 1 CNGB1a, cone photoreceptor contains 2 CNGA3 and 2 CNGB3, whereas channels in olfactory neurons are composed of 2CNGA2, 1 CNGA4, and 1 CNGB1b. In parallel to HCN channels, the C-terminal contains a cyclic nucleotide-binding domain. Photoreceptors have strong preference for cGMP, whereas the olfactory channel is almost equally sensitive to both ligands. B subunits do not form functional channels but modulate the channel properties of A subunits. As with other channels, differential splicing of primary transcripts

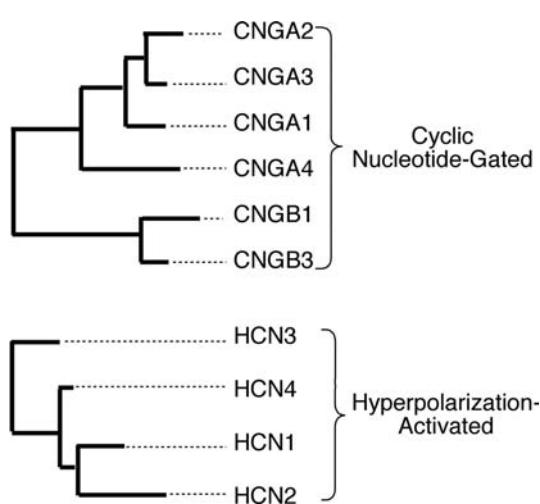


Fig. 22. Phylogenetic tree of cyclic nucleotide-modulated ion channels. (Derived from F.H. Yu and W.A. Catterall, Science STKE 2004; 253:re15.)

yields channels of altered structure and behavior. The channels are permeable to Na^+ , K^+ , and Ca^{2+} , but not to Cl^- and other anions. CNG channels are not voltage sensitive, but their current-voltage curves show an outward rectification. This is a result of activation of Ca^{2+} binding sites in the pore of the channel. In physiologic extracellular concentrations, Ca^{2+} binds to this site, resulting in a decrease in conductivity. Other divalent cations mimic the action of Ca^{2+} .

3.6. TRP Ion Channels in the Nervous System

The transient receptor potential (TRP) channels were initially discovered in *Drosophila*, where they contribute to phototransduction. They represent the second largest family of voltage-gated ion channels. TRP channels are six-TM spanning segment proteins, with the pore domain between the fifth and sixth segments. Thus, these channels resemble K_v channels in overall structure (Fig. 17). However, they show limited conservation of the S4 positive charges and P loop sequences. Assembly of channel subunits as homo- and heterotetramers results in formation of cation-selective channels. Their activation leads to slow depolarization of cells and rise in intracellular Na^+ and Ca^{2+} , with the exceptions of two members, which are only permeable to monovalent ions. TRPs are classified into at least six distinct subfamilies (Fig. 23). The “canonical” TRPC family is composed of three subgroups, and these channels are most closely related to *Drosophila* TRP channels. Many of TRPC channel subunits are able to coassemble. They are usually activated by receptors coupled to phospholipase C signaling pathway and may contribute to the sustained elevation in intracellular Ca^{2+} . The details of the activation mechanism remain elusive. It has long been proposed that TRPC channels underlie the store-operated channels observed in many cell types. As discussed in Section 6.3, two other proteins, stromal interaction molecules (STIMs) and the ORAIIs, have more recently been implicated in this process.

The TRPV channel family contains six members in mammals. TRPV1 to 4 are all heat-activated channels that also function as chemosensors and are nonselective for cations. The vanilloid receptor subtype 1 (TRPV1) is a nonselective ligand-gated cation channel that may be activated by a wide variety of exogenous and endogenous stimuli, including heat greater than 43°C , low pH, anandamide, and capsaicin. TRPV1 receptors are found in the CNS and the peripheral nervous system and are involved in the

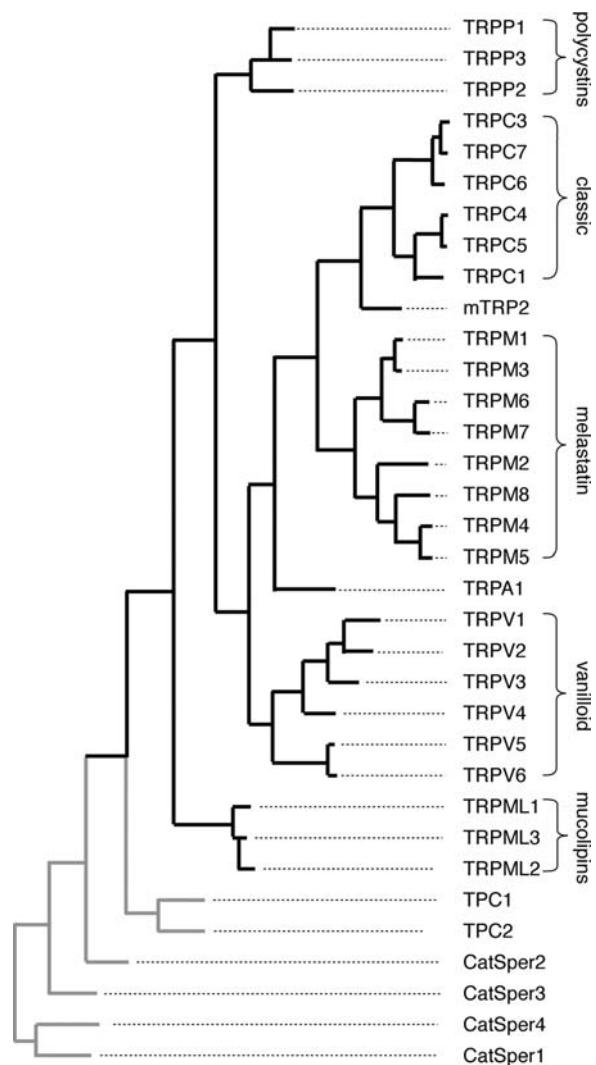


Fig. 23. Alignment of human TRP family of proteins (full black lines). The related TPC and CatSper channels are shown in full gray lines. (Derived from Clapham et al, 2005, Pharmacol Rev 57:427; and F.H. Yu and W.A. Catterall, Science STKE 2005; 253:re15.)

transmission and modulation of pain, as well as the integration of diverse painful stimuli. TRPV2 is immunolocalized to hypothalamic nuclei, preferentially in oxytocinergic and vasopressinergic neurons. TRPV3 is expressed widely but most strikingly in skin. TRPV4 may function as an osmo-transducer in primary afferent nociceptive nerve fibers. The properties of the two other channels from this subfamily, TRPV5 and TRPV6, are quite different. Under physiologic conditions, these channels (originally named ECAC and CAT1) exclusively conduct Ca^{2+} , and they are responsible for calcium absorption in kidney and intestine. Also, the temperature sensitivity of these two channels is low.

The TRPM subfamily has eight members divided into four groups. Unlike the TRPC and TRPV subfamilies, TRPM subunits do not contain N-terminal ankyrin repeats but contain entire functional proteins in their C-termini. The exceptional structural feature of these channels is the presence of functional enzymes in their C-terminal. TRPM2 contains a functional NUDT9 homology domain exhibiting ADP-ribose pyrophosphatase activity, whereas TRPM6 and TRPM7 contain functional α -kinase segments, which are a type of serine/threonine-specific protein kinase. The relative permeability of Ca^{2+} and Mg^{2+} varies widely among TRPM channels. TRPM4/5 have similar characteristics, including impermeability to Ca^{2+} , whereas TRPM3/6/7 are highly permeable to both Ca^{2+} and Mg^{2+} . The method of activation also varies greatly among TRPM channels. TRPM2 is highly expressed in the brain and is activated by adenosine diphosphate ribose and heat. TRPM4/5 are activated by intracellular calcium and heat. TRPM8 can be activated by low temperatures, menthol, eucalyptol, and icilin. Among the functional responsibilities of the TRPM channels are sensory transduction in taste cells (TRPM5) and regulation of magnesium reabsorption in the kidneys and absorption in the intestines (TRPM6).

The three members of the TRPML subfamily are not well characterized. TRPML1 is probably localized in late endosomes. This subunit also contains a lipase domain between its S1 and S2 segments. Although the function of this domain is unknown, it has been proposed that it is involved in channel regulation. Physiologic studies have described TRPML1 channels as proton leak channels in lysosomes responsible for preventing these organelles from becoming too acidic. TRPML2 and TRPML3, the latter expressed in cytoplasm of hair, have yet to be characterized.

TRPP subunits are very inhomogeneous and can be divided into two subcategories depending on structural similarity. The first group, TRPP1-like, contains TRPP1, PKDREJ, PKD1L1, PKD1L2, and PKD1L3. TRPP1 consist of 11 TM segments and contains numerous N-terminal adhesive domains that are important for cell-cell contact. This group of subunits also contains a large extracellular domain with numerous polycystin motifs. These motifs are of unknown function and are located between the S6 and S7 segments. The large intracellular C-terminal segment of TRPP1 seems to interact with TRPP2 to act as a signaling complex. The other group of TRPP members are TRPP2-like: TRPP2, TRPP3, and TRPP5. These channels resemble other TRP channels, having

6TM-spanning segments with intracellular N- and C-termini. TRPP2 and TRPP3 form constitutively active cation-selective ion channels that are permeable to Ca^{2+} . TRPP3 has also been implicated in sour taste perception. Coupling of TRPP1 and TRPP2 recruits TRPP2 to the membrane.

The sole member of the TRPA subfamily, TRPA1, contains 14 N-terminal ankyrin repeats and probably functions as a mechanical stress sensor. This channel is expressed in dorsal root ganglion, trigeminal ganglion neurons, and hair cells. There is conflict information about activation of this channel by noxious cold, which was not settled using TRPA1 knockout mice. Chemical activators of these channels are isothiocyanates, methyl salicylate in wintergreen oil, and cinnamaldehyde in cinnamon.

There are two groups of channels that are somewhat related to TRP channels and more distantly to Na_v and Ca_v channels: the *two-pore cation (TPC) channels* and *CatSper channels* 1 to 4 (Fig. 23). TPC channels are putative cation-selective channels that have two repeats of a six-TM domain. Each domain has a positively charged voltage sensor segment (Fig. 17). They are expressed in kidney, liver, and lung but could not be expressed in heterologous cells. CatSpers are putative 6TM voltage-gated and Ca^{2+} -permeant channels that appear to be specific to sperm cells.

3.7. Chloride Channels Also Tend to Reduce Excitation

The common characteristic of K^+ and Cl^- ions in neurons is their negative equilibrium potential; thus, activation of channels conducting these ions draws the membrane potential closer to their equilibrium potentials and farther from the threshold for firing. Channels conducting these ions tend to stabilize the membrane potential by setting the resting potential, repolarize and hyperpolarize cells after a depolarizing event, and control the interspike interval. They also control other cellular functions. Chloride channels are a functionally and structurally diverse group of ion channels. Excluding the ligand-gated γ -amino butyric acid (GABA) and glycine receptor channels (see Section 4.2), chloride channels can be classified as voltage-sensitive channels, calcium-activated channels, high maxi conductance channels, the cystic fibrosis transmembrane conductance receptor (CFTR), and volume-regulated channels.

Voltage-gated Cl^- (CIC) channels do not share structural similarity with K_v , Na_v , and Ca_v . So-called *Torpedo channels* were the first chloride channels to be cloned and termed CIC-0. The mammalian

versions of this channel, called CIC-1 to CIC7, ClC-K_a and ClC-K_b, have subsequently been identified. Alternate splicing increases the structural diversity within the ClC family of channels. All ClC channels have high chloride selectivity and show a voltage-dependent block by iodine. A detailed sequence comparison of ClC has revealed three closely related groups: the first group contains ClC-1, ClC-2, and the renal ClC-K-1 and ClC-K-2, the second group comprises ClC-3, ClC-4, and ClC-5; and the third group has two members, ClC-6 and ClC-7 (Fig. 24A). All ClC proteins (~700 to 1000 Da) are predicted to contain 10 to 12 TM domains with intracellular N- and C-termini (Fig. 24B). Except ClC-1 and ClC-K1/K2, which are specific for kidney, most other ClC are widely distributed in various tissues. It is likely that these channels have different functions in various tissues and cells.

Calcium-activated Cl⁻ (CaCC) channels are widely expressed in excitable and nonexcitable cells, including neurons. Numerous calcium-activated chloride channel-like proteins have been cloned. However, their relationship with endogenous CaCC remains to

be established. *Maxi Cl⁻ channels* are high-conductance and anion-selective channels that were initially characterized in skeletal muscle. Subsequently, these channels were found in neurons, glial cells, cardiac muscle, and other cell types. The structure and physiologic significance of these channels is uncertain. The cystic fibrosis transmembrane conductance receptor (CFTR) is a 12-TM epithelial cell membrane Cl⁻ channel that is regulated by PKA. These channels are involved in normal fluid transport across various epithelia. The most common mutation in this protein results in impaired trafficking of CFTR causing cystic fibrosis. *Volume-activated chloride channels* participate in regulation of volume decrease in response to cell swelling. These channels may also contribute to the regulation of membrane excitability and other cellular functions. At the present time, their structure is unknown, but several reports indicated that the members of ClC proteins might form these channels.

4. EXTRACELLULAR LIGAND-GATED CHANNELS

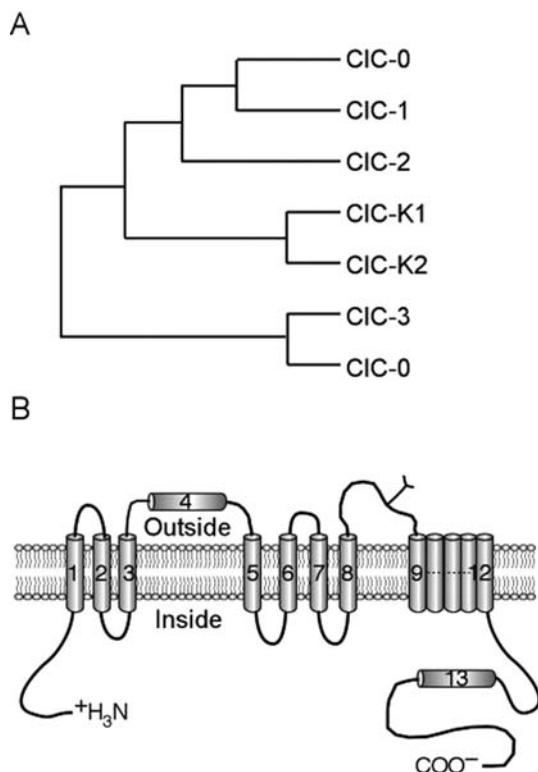


Fig. 24. Voltage-gated chloride channels. (A) Dendrogram describing the relationship between different members of the ClC family of channels. (B) Putative transmembrane organization of ClC channels. (Derived from T.J. Jentsch, W. Gunter, M. Pusch, and B. Schwappach, J Physiol 1995; 482P:19S.)

The activation of *ligand-gated receptor channels* depends on the delivery and binding of a ligand to the extracellular domain of these receptor channels. Because ligand-gated channels are generally activated by neurotransmitters, they are also known as *neurotransmitter-controlled channels*. Termination of activities of these channels requires removal of the ligand, which is usually mediated by a specific pathway for ligand degradation and/or uptake. During prolonged occupancy of receptors, the conductance through ligand-gated receptor channels decreases in a process called *desensitization*, which is analogous to inactivation of voltage-gated channels. At the single-channel-level recordings, desensitization corresponds with the closure of channels during steady agonist application. Desensitized channels are unable to respond to added neurotransmitter but recover their sensitivity after the agonist is removed. The rates of desensitization and recovery are receptor-specific. The molecular mechanism and physiologic importance of desensitization are not fully characterized. Interestingly, many ligand-gated channels share common agonists with G protein-coupled receptors (see Chapters 4 and 5).

There are two classes of ligand-gated channels, the *excitatory cation-selective channels*, operated by acetylcholine, glutamate, 5-hydroxytryptamine (5-HT), and adenosine 5'-triphosphate (ATP), and the *inhibitory anion-selective channels*, activated by GABA and

glycine. Structural information obtained by cDNA cloning of ligand-gated receptor channels has led to the identification of three families of evolutionary-related proteins. The 5-HT₃, GABA, and glycine receptor channels possess structural features similar to the nicotinic acetylcholine-activated receptor channel, and they are grouped as one family, known as ligand-gated ion channels of the *Cys-loop family*. These channels are composed of five subunits (pentamers), each of which contributes to the ionic pore. All subunits have a large extracellular N-terminal region followed by four hydrophobic putative membrane-spanning segments and an extracellular C-terminus (Fig. 25A). Glutamate-activated receptor channels are composed of four TM segments, but their M2 segment forms a pore-loop structure, entering and exiting the cell membrane from the intracellular side. Thus, the N-terminus is extracellularly located, whereas the C-terminus is intracellularly located and is regulated by signaling molecules, including the kinases (Fig. 26A). A detailed analysis of the intra-subunit interactions that govern glutamate-receptor assembly indicates that these channels are dimers of dimers. The ATP-gated (purinergic) receptor channels have only two putative TM domains with the N- and C-terminus facing the cytoplasm (Fig. 27A). As with nicotinic and glutamate channels, the functional diversity of P2X channels is generated by subunit multimerization. The functional channels are probably composed of three subunits.

4.1. Nicotinic Acetylcholine and 5-Hydroxytryptamine Receptors Are Cation-Permeable Channels

The native acetylcholine-activated receptor channel (nAChR) was initially identified as a pentamer protein of about 300,000 MW from the fish electrical organ. Recombinant DNA studies revealed close homologies between nAChR subunit sequences derived from electrical organ and skeletal muscle tissue, as well as between muscle and neuronal nAChRs. Genes encoding a total of 17 subunits have been identified: peripheral nAChR subunits, labeled as α_1 , β_1 , δ , γ , and ϵ , and neuronal nAChR subunits, labeled as α_2 to α_{10} and β_2 to β_4 . In both muscle and neuronal nAChRs, the large N-terminal domain contains the ligand-binding sites. The second TM segment forms the wall of ion channel, and the variable C-terminal domain faces the cytoplasm and is subject to regulation by phosphorylation (Fig. 25A). Both peripheral and neuronal receptors form heteropentamers that form barrel-like structures. In muscle and

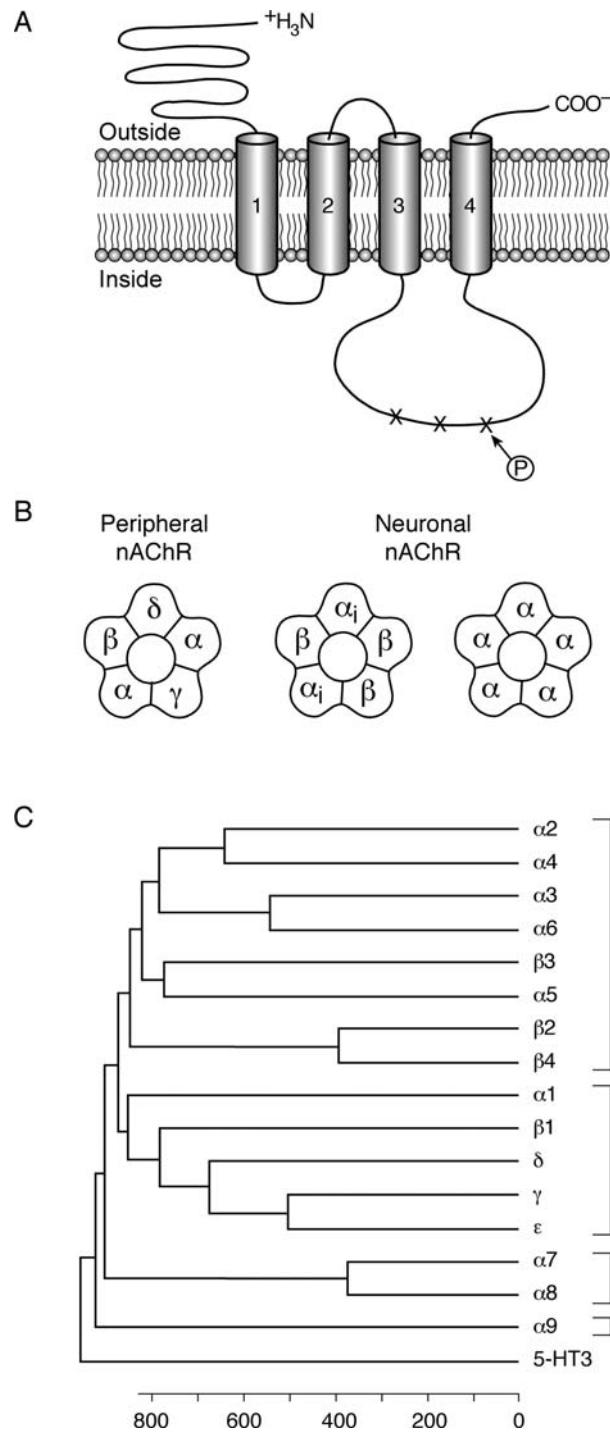


Fig. 25. Structural organization of muscle and neuronal nicotinic channels (nAChRs). (A) Putative transmembrane organization of nAChRs. X, the potential phosphorylation sites. (B) Front view of the models of muscle and neuronal nAChRs. (C) Simplified dendrograms of the members of the nicotinic receptor channel family. (Derived from Changeux JP, Brain Res Rev 1998; 26:198.)

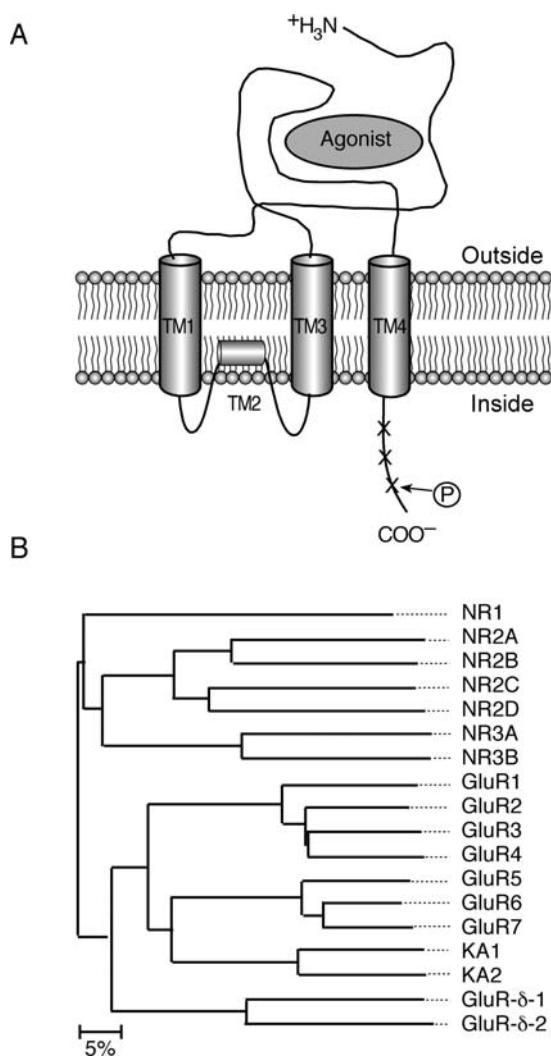


Fig. 26. Structural organization of glutamate receptor channels. (A) TM topology of glutamate ion channels. TM, transmembrane domain. TM2 does not cross the membrane, although it contributes to the lining of the pore. X, the potential phosphorylation sites. (B) Simplified dendrograms of the members of the glutamate receptor channel family. (Derived from J.N. Kew and J.A. Kemp, Psychopharmacology 2005; 179:4.)

electrical organs, nAChRs are composed of four subunits: α , β , γ or ϵ , and δ . The neuronal AChRs assemble according to the general $2\alpha + 3\beta$ stoichiometry, with possibly more than one α -subunit class within a pentamer. Figure 25B illustrates the models for peripheral and neuronal nAChRs. The phylogenetic trees of these subunits are shown in Figure 25C. In reconstitution experiments, neuronal subunits also form functional homo-oligomeric channels. In addition, multiple combinations of α and β subunits from two or more different subtypes form a wide range of functional hetero-oligomers.

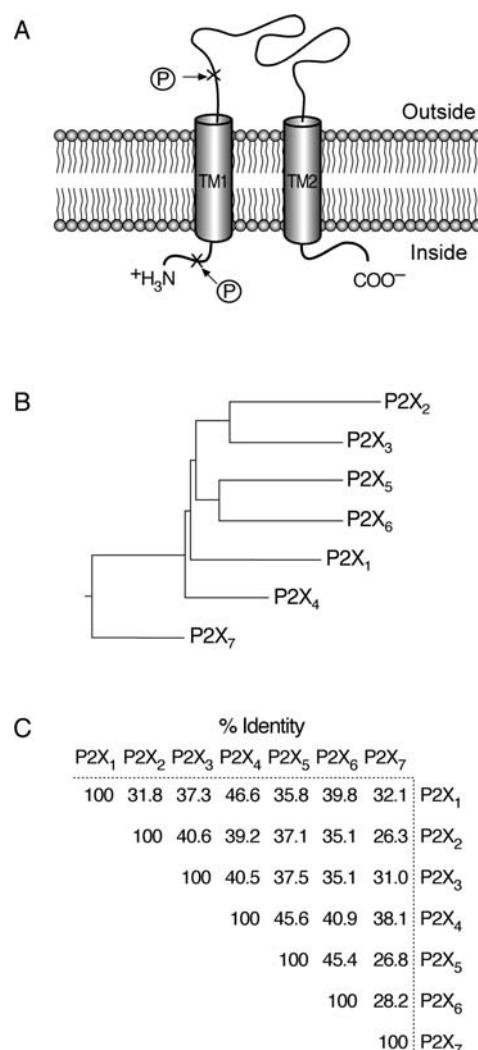


Fig. 27. Structural organization of cation-conducting purinergic-receptor channels. (A) Topological model of purinergic-receptor channels. X, the potential phosphorylation sites. (B) The relationship among seven P2X subunits. (C) Percentage identity of seven rat P2X subunits. (Derived from B.S. Khakh, G. Burnstock, C. Kennedy, B.F. King, R.A. North, P. Seguela, M. Vaigt, and P.P. Humphrey, Pharmacol Rev 2001; 53:107.)

These multiple combinations of nAChR subunits possess distinct pharmacologic and physiologic properties. The muscle nAChRs are the least permeable to Ca^{2+} , and the estimated percentage of the inward current carried by Ca^{2+} is only 2%. Neuronal nAChRs exhibit a higher permeability to Ca^{2+} than do muscle and electrical organ receptors. This is because of the multiple combinatorial possibilities available for the assembly of the various subunits into hetero-oligomers. For example, the α_7/α_8 subunit-based homo-oligomers are highly permeable for Ca^{2+} compared with Na^+ , whereas subunits α_2 to α_6

and β_2 to β_4 from hetero-oligomeric channels have similar permeabilities for Ca^{2+} and Na^+ . The specific amino acids important for Ca^{2+} permeability and selectivity of neuronal nAChRs are located in the M2 domain. Also, the subunit composition determines the rate of desensitization. Furthermore, the distribution of neuronal nAChR subunits within the brain of adult animals varies. The expression of these subunits during embryonic and postnatal development is highly specific for a particular subunit.

The nAChRs are critical for neuromuscular coupling. Upon binding of acetylcholine, nAChR channels open to allow Na^+ to flow through the channel. The resulting plasma membrane depolarization opens Ca_v channels and initiates Ca^{2+} release from sarcoplasmic reticulum through ryanodine receptor channels (see later). In skeletal muscle, activation of voltage-gated Ca^{2+} influx in the T-tubule plasma membrane is the primary signal that activates intracellular Ca^{2+} release channels and ultimately stimulates muscular contraction. Although the nAChRs are found in most parts of the brain, their functional significance is not well-characterized. Both Ca^{2+} influxes through activated nAChRs and Ca^{2+} potentiation probably account for the physiologic actions of these channels. Calcium potentiation is a process in which Ca^{2+} influx through one channel regulates the efficacy of other ligand-gated channels, leading to the modulation of membrane excitability in neurons, as well as their ability to integrate synaptic and paracrine signals. Furthermore, nAChR-dependent Ca^{2+} signals enhance protein-kinase activity in myotubes, leading to phosphorylation of the nAChR γ subunit. Because this process is dependent on Ca^{2+} influx, it can be considered as autoregulation of phosphorylation by nAChRs. Recent results indicate that point mutation in this receptor may abolish desensitization, increase the affinity for agonists, and convert the effects of competitive antagonists into the agonist responses. Such mutations also occur spontaneously in humans and may be involved in diseases such as congenital myasthenia or frontal-lobe epilepsy.

The 5-hydroxytryptamine (5-HT₃) receptors also exist as pentamers and form cation-selective channels. The 5-HT₃ receptor is expressed throughout the central and peripheral nervous systems and mediates a variety of physiologic functions. This includes fast excitatory synaptic transmission in neocortical interneurons, amygdala, and visual cortex. These receptor channels are also present on presynaptic nerve terminals, where they contribute to the control of neurotransmission. Three 5-HT₃

subunits (5-HT_{3A}, 5-HT_{3B}, and 5-HT_{3C}) have been cloned but only homomeric 5-HT_{3A} and heteromeric H-HT_{3A+3B} form functional receptors when expressed in heterologous systems. A short form of 5HT_{3A} receptor subunit was also identified, but this splice form does not differ from the wild-type channel. Homomeric and heteromeric channels mediate a rapidly activating, desensitizing, inward current predominately carried by sodium and potassium ions. The channel is formed from the second TM segment.

4.2. GABA and Glycine Receptors Are Anion-Permeable Channels

γ -Amino butyric acid (GABA) is a major inhibitory transmitter in the vertebrate CNS that acts through two different receptor channels, GABA_A and GABA_C, in addition to G protein-coupled GABA_B receptors (see Chapter 5). In immature neurons, however, GABA-mediated responses are often depolarizing, caused by Cl^- efflux due to the high intracellular Cl^- concentration (see Section 7). GABA_A receptor subunits show 20% to 30% sequence identity with AChR, glycine receptor, and 5-HT₃ receptor. Several subunit classes and isoforms within each class of the GABA_A receptor have been cloned, including $\alpha 1$ to $\alpha 6$, $\beta 1$ to $\beta 3$, $\gamma 1$ to $\gamma 3$, δ , ϵ , π , and $\rho 1$ to $\rho 3$. Various isoforms of the ρ subunits are the major molecular components of GABA_C receptors. Within these families, additional variants arise through alternative splicing. The expression of these subunits varies within the brain. GABA_A receptor is a pentameric assembly derived from a combination of various subunits. The preferred combination includes two α , two γ , and one β subunit. However, the colocalization of these three types of subunits is not an absolute requirement for the formation of functional channel. The great diversity of receptor subunits leads to profound differences in tissue distribution, ontogeny, pharmacology, and regulation of GABA_A receptors. These receptors are targets for many drugs in wide clinical use, including benzodiazepines, barbiturates, neurosteroids, ethanol, and general anesthetics, which increase the conductance through GABA channels. Bicuculline inhibits GABA_A but not GABA_C channels. At the single-channel level, barbiturates increase the opening time of channels, whereas benzodiazepines increase the number of channel openings.

Glycine is the other main inhibitory neurotransmitter in the CNS, particularly in the spinal cord and in the brain stem, whereas GABA is more abundant in

rostral parts of the CNS. The *glycine receptors* (GlyRs) are also pentameric proteins composed of three α and two β subunits. There are four isoforms of α subunits, which have highly homologous sequences but different pharmacologic and functional properties. GlyRs heterogeneity is further increased by alternative splicing of α subunits. The α subunit contains the ligand-binding site and is sufficient to form functional homomeric channel, whereas β subunit modulates the pharmacologic and conductance properties of the GlyRs. The TM2 segment generates an ion-permeable pore. Both α and β subunits contain recognition motifs for various protein kinases, including PKA and PKC and tyrosine kinases. At synapses, GlyRs are clustered at the postsynaptic membrane directly opposite the presynaptic release sites and are linked to the subsynaptic cytoskeleton by a membrane protein named gephyrin. There are many similarities between GABA_A and GlyRs, including ion selectivity, which arise from their close and conservative evolutionary relationship, and both channels are more distantly related to nAChRs and 5-HT₃ receptors.

4.3. Some Glutamate Channels Are Voltage- and Ligand-Gated

L-Glutamate is the major excitatory neurotransmitter in the CNS, and it acts through a variety of ionotropic and metabotropic receptors. *Ionotropic glutamate-activated receptor channels* (iGluRs) differ from the four TM domain model of nAChRs. They possess an extracellular N-domain, followed by the first TM domain and then the pore-forming membrane-residing domain that does not cross the membrane but forms a loop. Specific residues in this segment determine the ion selectivity of the channel. The 3TM and 4TM domains are linked by a large extracellular loop, followed by an intracellular C-domain (Fig. 26A). iGluRs are encoded by 18 genes that assemble to form four major subtypes: the α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate, *N*-methyl-D-aspartate (NMDA), and delta receptor channels. Molecular cloning has revealed several subunits for each receptor group. Four AMPA receptor genes (GluR1 to GluR4) denote the AMPA-sensitive family, whereas five kainate receptor genes (GluR5 to GluR7, plus KA1 and KA2) denote the kainate subclass. For NMDA-receptor channels, seven subunits (NR1, NR2A to NR2D, NR3A, and NR3B) have been established. In addition, two δ subunits also exist

and belong to the GluR-type subunit, but the function of this particular subunit is unknown. The phylogenetic trees of these subunits are shown in Fig. 26B. Finally, the molecular diversity of NR1 and GluRs is further increased by variants created by alternative splicing and RNA editing.

In addition to the specific structure and pharmacology, NMDA channels exhibit a different excitation behavior than those not activated by NMDA. These channels are both ligand- and voltage-gated. Full activation of the NMDA receptor requires application of two ligands, L-glutamate and glycine. The NMDA receptors only become fully activated by glutamate after their Mg²⁺ block has been relieved by membrane depolarization. The NMDA receptor exhibits low-binding-affinity sites for Ca²⁺, which results in a low selectivity among cations. However, because of the lower-affinity binding, Ca²⁺ moves through the pore rapidly. Their kinetics is much slower, resulting in a large Ca²⁺ influx and long-term metabolic or structural changes. The importance of Ca²⁺ signals generated by NMDA receptor channel activity is also well-established. Some of the most important functions of the nervous system, such as synaptic plasticity, are dependent on the behavior of NMDA receptor channels and Ca²⁺ influx through these channels.

Kainate and AMPA receptor subunits do not form mixed channel complexes, but both types of receptors can be expressed in the same neuron. Native AMPA receptors are either homomeric or heteromeric oligomers composed of these multiple subunits. AMPA receptors in mammalian CNS differ considerably with respect to gating kinetics and Ca²⁺ permeability. Although AMPA channels were generally considered to be permeable only to Na⁺ and K⁺, some native AMPA receptors display a substantial permeability to Ca²⁺ and a weaker selectivity among the divalent cations compared with NMDA channels. The rapid kinetics of AMPA receptors is suitable for rapid neurotransmission. The Ca²⁺ permeable AMPA receptors are involved in the excitatory synaptic transmission in hippocampal and neocortical nonpyramidal neurons. It is believed that Ca²⁺ influx through these channels plays a significant role in modulating the long-term synaptic functions. A number of recent studies have also indicated a role of kainate receptors at neuronal synapses. Both presynaptic and postsynaptic localization of these receptors has been suggested. Depending on the subtype of receptors and the localization, these channels may exhibit stimulatory or inhibitory action.

4.4. ATP-Gated Channels Are Expressed in Excitable and Nonexcitable Cells

With the use of molecular cloning techniques, seven *purinergic receptor channel* (P2XR) subtypes have been identified to date—denoted as P2X₁R through P2X₇R—and several spliced forms have also been observed. P2XR subtypes differ with respect to their ligand-selectivity profiles, antagonist sensitivity, and cation selectivity. They can form ion-permeable pores through homo- and heteropolymerization. Each subunit is proposed to have two TM helices connected by a large extracellular loop, with both N- and C-termini located in the cytoplasm (Fig. 27A). From the N-termini through the second TM domain, the cloned subunits exhibit a relatively high level of amino acid sequence homology (Fig. 27B, C). In contrast, the C-termini vary in length and show no apparent sequence homology, except for the region nearest to the second TM domain.

In addition to ligand-selectivity profiles and antagonist sensitivity of P2XRs, they also differ with respect to their desensitization rates. Based on the observed differences in their current and calcium desensitization kinetics, homomeric P2XRs are generally divided into three groups: P2X₁R and P2X₃R desensitize very rapidly, P2X₄ desensitizes at a moderate rate, whereas P2X₂R, P2X₅R, and P2X₇R show little or no desensitization. Heteropolymerization results in channels that desensitize with a pattern different from those seen in cells expressing homomeric channels. Native P2XRs also desensitize with different kinetics, which reflect their structure. The differences in desensitization rates of P2XRs are reminiscent of those seen among subtypes of other ligand-gated receptor channels. Site-directed mutagenesis experiments indicated the relevance of C-terminus structure on P2XR desensitization patterns and have identified the region around Arg³⁷¹ as important in this process. Calcium and PKC may also play a role in control of receptor desensitization and recovery from desensitization through still uncharacterized pathway(s). A dual control of receptor-channel function resembles that of voltage-gated channels.

In contrast with the well-characterized structure and pharmacology of P2XRs, the understanding of their physiologic significance is still in progress. In general, Ca²⁺ is a charge carrier through these channels, although the permeability of Ca²⁺ versus Na⁺ varies widely among different cell types. Thus, these channels can serve as Ca²⁺ influx channels. These channels also facilitate Ca²⁺ influx indirectly, by

depolarizing cells and activating Ca_v. In addition to stimulating intracellular Ca²⁺ signals, the paracrine actions of ATP on purinergic receptors can generate the cell-to-cell spread of Ca²⁺ signals in glial cells in the absence of gap-junctional communication. The best-characterized agonist role of ATP is in synaptic transmission from sympathetic nerves, where ATP acts as a co-transmitter with noradrenaline. ATP has also been implicated in parasympathetic-, sensory-, and somatic-neuromuscular transmission. About 40% of hypothalamic neurons in culture respond to ATP by a rapid increase in intracellular Ca²⁺ because of Ca²⁺ entry through P2XRs. Purinergic receptor channels are expressed in neurons, as well as in other excitable cells, including pituitary cells, and nonexcitable cells, including gonadal cells and lymphocytes.

5. ENaC/DEGENERIN FAMILY OF CHANNELS

The recently discovered *epithelial sodium channel* (ENaC)/*degenerin* (DEG) gene superfamily encodes Na⁺ channels involved in various cellular functions. This superfamily of channels include ENaC, acid-sensing ion channels (ASICs), FMRF-amide activated channels of *Helix aspersa*, DEG of *Caenorhabditis elegans*, and orphan channels. The members of ENaC/DEG gene superfamily show a high degree of functional heterogeneity not observed among voltage-gated ion channels. These channels are constitutively active (ENaC), activated by mechanical stimuli (DEG) or by ligands such as peptides (FMRF) and protons (ASICs). The topology of ENaC/DEG proteins comprises N- and C-terminals located intracellularly, two hydrophobic TM domains, and a large extracellular loop. As with P2X receptors, ENaC/DEG family members contain conserved cysteine-rich domains, which might be involved in the formation of disulfide bonds needed for the proper tertiary structure of channels. Functional channels are homo- and heteromers.

In contrast with Na_v channels, ENaC is localized in apical membrane of polarized epithelial cells, where it mediates Na⁺ transport across tight epithelia. The ENaC-mediated reabsorption of Na⁺ occurs in the epithelial lining of the distal part of the kidney tubule, the alveolar epithelium, and the distal colon epithelium. There is an overall 37% amino acid identity among α , δ , β , and γ subunits. Both α and δ subunits can form independent conducting channels, whereas β and γ subunits are modifying subunits that regulate the trafficking and the conductance of the

ion pore-forming subunits. Similar to other ENaC/DEG superfamily members, ENaC is very sensitive to amiloride.

Acid-sensitive cation channels (ASICs) are directly activated by a drop in extracellular pH. Six different ASICs subunits encoded by four genes have been cloned from neuronal tissue: ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4. The first four subunits form functional channels when expressed in heterologous systems, whereas ASIC4 cloned from pituitary tissue does not produce a proton-gated channel. The recombinant channels generate proton-gated currents with functional properties resembling the native currents. The functional role of ASICs in the peripheral nervous system was linked with nociception, perception of sour taste, modulation of synaptic transmission, and mechanosensory transduction. These channels are also expressed in the brain, but their function in the central neurons is still not clarified. ASIC2 knockout mice had normal appearance, growth, fertility, and life span and showed no obvious defects in pain sensation.

6. INTRACELLULAR AND INTERCELLULAR CHANNELS

The expression of ion channels is not limited to the plasma membrane. Two types of channels, *ryanodine receptor channels* (RyRs) and *inositol 1,4,5-trisphosphate-receptor channels* (IP₃Rs), are expressed in the ER/sarcoplasmic reticulum membrane and nuclear membrane. RyRs provide an effective mechanism for transduction and translation of electrical signals inside of cells, whereas IP₃Rs are activated by two classes of plasma-membrane receptors known as *Ca²⁺-mobilizing receptors*. Their activation is independent of the electrical status of cells and represents the major pathway for Ca²⁺ signaling in nonexcitable cells. However, this signaling pathway is also operative in excitable cells, including neurons. Activation of IP₃Rs leads to stimulation of voltage-insensitive Ca²⁺ channels expressed on the plasma membrane, and this process is known as *capacitative Ca²⁺ entry*. Channels that accommodate capacitative Ca²⁺ entry are also expressed in both excitable and nonexcitable cells.

6.1. Voltage-Gated Ca²⁺ Influx Activates Ryanodine Receptors

RyRs were originally identified as Ca²⁺ release channels expressed in the sarcoplasmic reticulum of

skeletal muscle fibers and cardiac myocytes, where they play a central role in excitation-contraction coupling. These channels are also expressed in neurons, chromaffin cells, sea urchin eggs, and several nonexcitable cell types. Mammalian tissues express three isoforms: RyR₁ is expressed predominately in skeletal muscle, RyR₂ is expressed in cardiac muscle, and RyR₃ has a wide tissue distribution, including the nonexcitable cells. RyR₁ and RyR₂ channels display a 66% identity, whereas the RyR₃ channel is much shorter. RyRs are tetramers, with a large N-terminal region forming heads, and a C-terminal region that forms the Ca²⁺-selective channel. Although these channels are frequently coexpressed with IP₃Rs, the physiologic importance of their coexpression and their variable density within the cells are still largely unknown.

RyRs are the largest known ion channels and are susceptible to many different modulators, including cytosolic calcium, membrane potential, and several intracellular messengers. As in the regulation of IP₃Rs, intracellular Ca²⁺ is a major regulator of RyRs; at low concentrations, Ca²⁺ promotes release, whereas higher concentrations are inhibitory. However, the inhibition of RyRs by high intracellular Ca²⁺ concentration is somewhat controversial, because it requires intracellular Ca²⁺ to be in the millimolar concentration range, which is not reached under physiologic conditions. The ability of Ca²⁺ to stimulate its release from the endoplasmic/sarcoplasmic reticulum via RyRs is known as *Ca²⁺-induced Ca²⁺ release*. This process is of fundamental importance for coordinating the elementary Ca²⁺-release events into Ca²⁺ spikes and waves. Unlike IP₃Rs, RyRs can release Ca²⁺ in response to an increase in intracellular Ca²⁺ concentration with no other change in the concentration of second messengers. This is crucial for excitation-contraction coupling. For example, in cardiac cells, Ca²⁺ entry through dihydropyridine-sensitive channels activates RyRs to induce a further increase in intracellular Ca²⁺. In skeletal muscle cells, the dihydropyridine receptors act primarily as voltage sensors to directly activate RyRs in response to membrane depolarization.

In addition to Ca²⁺, there are numerous other endogenous modulators of RyRs. The best-known is ryanodine, which activates and inhibits RyRs, depending on its concentrations. At 1 to 10 μM concentration, ryanodine locks the channel in a subconductance stage that slows the opening and closing of the channel. At higher concentrations, ryanodine inhibits RyRs, and this action is mimicked by ruthenium red. Other modulators

are endoplasmic/sarcoplasmic reticulum Ca^{2+} , cytosolic pH, Mg^{2+} and other cations, Cl^- and other anions, nucleotides, cyclic adenosine 5'-diphosphate ribose, several protein kinases, calmodulin, and other Ca^{2+} -binding proteins. Caffeine is a standard pharmacologic tool for the activation of RyRs. It acts on RyRs in both intact cells and isolated channels. Another pharmacologic agent, dantrolene, has biphasic effects on RyR₁; in nanomolar concentrations it increases the open probability of these channels, and in micromolar concentrations it inactivates the channel.

6.2. IP_3 Receptors Are Intracellular Ligand-Gated Channels

Activation of IP_3 Rs is triggered by seven membrane-domain receptors coupled to G proteins and tyrosine kinase plasma-membrane receptors. Calcium-mobilizing receptors that are coupled to G_q/G_{11} , as well as several receptors coupled to G_s and G_i , activate phospholipase C- β , whereas tyrosine kinase receptors activate phospholipase C- γ . Both enzymes hydrolyze the membrane-associated phosphatidylinositol 4,5-bisphosphate to increase the production of IP_3 and DAG. IP_3 rapidly diffuses into the cytosol to activate IP_3 Rs. In contrast, DAG remains in the plasma membrane, where it acts on PKC.

IP_3 Rs are composed of four similar subunits that are noncovalently associated to form a four-leaf clover-like structure, the center of which makes the Ca^{2+} -selective channel. Each subunit contains ~2700 amino acids, with the cytoplasmic N-terminus comprising ~85% of the protein mass, a hydrophobic region predicted to contain six membrane-spanning helices, and a short cytoplasmic C-terminus. The IP_3 binding sites are located within the first 788 residues of the N terminus of each subunit. Complete cDNA sequences of three distinct IP_3 R encoding genes have been determined. Most cells express multiple isoforms of IP_3 Rs, indicating that they have different functions. Analysis of the single-channel function of type-1, type-2, and type-3 IP_3 R revealed isoform-specific properties in terms of their sensitivity to IP_3 and Ca^{2+} . Further diversity of IP_3 R expression is created by alternative splicing. IP_3 Rs are present in almost all cells and are localized in the ER membrane, nuclear membrane, and possibly the plasma membrane in some cell types. Functionally reconstituted purified IP_3 Rs respond to IP_3 , with an increase in the open probability resulting from a large conformational change. The release of Ca^{2+} is electrically compensated by an inward potassium flux.

Cytosolic Ca^{2+} is the major messenger that controls IP_3 R gating. In the presence of stimulatory concentrations of IP_3 , type-1 and type-2 IP_3 Rs respond to increases in intracellular calcium in a biphasic manner; Ca^{2+} increases IP_3 R activity at low concentrations and inhibits it at higher concentrations. Conversely, the type-3 IP_3 R open probability increases monotonically as the concentration of cytosolic Ca^{2+} increases. In both cases, the binding of IP_3 to residues within the N-terminal domain of IP_3 Rs is required for cytosolic Ca^{2+} to exhibit its messenger function. Like voltage-gated channels, several other factors also modulate the activity of IP_3 Rs, including PKA, PKC, calcium/calmodulin-dependent protein kinase II, adenine nucleotides, and pH. Two inhibitors, heparin and decavanadate, competitively inhibit IP_3 binding to IP_3 Rs, yet neither inhibitor is highly specific. Caffeine, a RyR stimulator, inhibits IP_3 Rs. A series of xestospongiins have been identified as non-competitive IP_3 R antagonists.

6.3. Calcium Release Is Coupled to Capacitative Ca^{2+} Entry

The term *capacitative Ca^{2+} entry*, by analogy with a capacitor in an electrical circuit, implies that intracellular Ca^{2+} stores prevent entry when they are charged (filled by Ca^{2+}) but promote entry as soon as the stored Ca^{2+} is discharged (released). The similarities in the properties of this entry within different cell types, including excitable cells, suggest a common mechanism. In addition to Ca^{2+} -mobilizing agonists, capacitative Ca^{2+} entry can be activated by injection of IP_3 or its nonmetabolized forms into the cell, inhibition of the Ca^{2+} pump by thapsigargin, discharge of the intracellular content by calcium ionophores, or prolonged incubation of cells in Ca^{2+} -deficient medium. Injection of heparin, an IP_3 R inhibitor, completely blocks agonist and IP_3 -induced Ca^{2+} mobilization and capacitative Ca^{2+} entry. Because depletion of the ER Ca^{2+} stores is followed by the influx of Ca^{2+} into the cell, the channels involved in such influx were termed *store-operated Ca^{2+} -selective plasma-membrane channels* (SOCCs). Until recently, it was believed that excitable cells do not express SOCCs. However, recent evidence suggests that neurons and other excitable cells also express SOCCs, and that these channels act as a Ca^{2+} influx pathway and as pacemaker channels to modulate action potential-driven Ca^{2+} entry. For example, depletion of the ER Ca^{2+} stores by the activation of Ca^{2+} -mobilizing receptors or thapsigargin activates SOCCs in N1E-115 neuroblastoma cells and

gonadotropin-releasing hormone (GnRH)-secreting neurons. Because of its depolarizing nature, I_{SOCC} functionally operates as a pacemaker current in GnRH neurons, opposing the hyperpolarizing action of voltage-gated potassium A-current. It is likely that SOCCs have dual action in neurons, to conduct Ca²⁺ and to facilitate voltage-gated Ca²⁺ influx.

At the present time, the nature of these channels and the mechanism of their regulation in response to store depletion are unknown. Several types of channels have been suggested to mediate capacitative Ca²⁺ entry: calcium release-activated (CRAC) channels, Ca²⁺-activated nonselective channels, TRP channels, and CaT1, a member of the “osm” channels. More recently, two proteins have been identified as critical for SOCC current: stromal-interacting molecule (STIM-1) and Orai1. STIM1 is a single spanning membrane protein and Orai is a tetra-spanning plasma membrane protein. STIM1 appears to be a “sensor” of Ca²⁺ within the ER Ca²⁺ store, moving in response to store depletion into ER puncta close to the plasma membrane. Suppression of STIM1 expression prevents store-operated activation of CRAC channels. STIM2 protein may also contribute to the control of capacitative Ca²⁺ entry. On the other hand, Orai1 itself forms the pore of SOCCs. There are three closely related and widely expressed Orai genes (Orai1, Orai2, and Orai3). Heteromeric combinations of these proteins may result in channels with distinct regulatory and/or coupling processes.

6.4. Electrical, Ca²⁺, and Chemical Coupling by Gap-Junction Channels

The cytoplasmic compartments of neighboring cells are frequently connected by *gap junctions*, which are clusters of intercellular channels that form a cytoplasmic bridge between adjacent cells to allow for the cell-to-cell transfer of ions, metabolites, and small messenger molecules, including Ca²⁺, ATP, cAMP, cADP ribose, and IP₃. Thus, gap-junction channels provide an effective mechanism for electrical, calcium, and metabolic coupling, depending on the size of the pore. Vertebrate intercellular channels are made up of a multigene family of conserved proteins called *connexins*. The invertebrate gap-junction channels have no detectable sequence homology with vertebrate gap junctions, although they exhibit similar functions and membrane topology. These channels are known as *innexins*. Recently, another family of junctional coupling proteins has been identified in

mammals. These channels are called *pannexins* and have low sequence homology, but general structure similarity, to a family of innexins.

To date, at least 20 connexin genes have been identified. Connexins are made up of four hydrophobic TM domains, with the N- and C-termini located in the cytoplasm. In the plasma membrane, six connexin subunits assemble in a circle to form hemichannels known as *connexons*, which can contain a single type of connexin (homomeric), or multiple connexins (heteromeric) to form the hemichannel pore. When two connexons from adjacent cells come together, they form an intercellular channel that spans the gap between the two cells. Two identical connexons or different connexons can join to form either homotypic or heterotypic intercellular channels, respectively. The presence of heteromeric connexins and heterotypic intercellular channels can produce a diverse group of structurally different intercellular channels, with different permeabilities and/or functions. A variety of other factors, including membrane potential, Ca²⁺, pH, and phosphorylation of channels, can also alter gap-junction channels. Several neurotransmitters and hormones, such as dopamine, acetylcholine, GABA, and estrogens, have also been found to alter intercellular channel activity. Pannexins are three-membered family of chemichannels (Panx1, Panx2, Panx3) that may also release ATP and couple to P2X₇ receptors.

Initially, gap junctions were detected as an electrical conductance between the presynaptic and postsynaptic elements. This type of synapse is termed *electrical synapse* to distinguish it from typical chemical synapse. Cell-to-cell connection through gap junction can be studied by electrophysiology, or under the fluorescent microscope by injecting the fluorescent dyes (Lucifer yellow, for example) into one cell and monitoring the diffusion of dye into its neighbors. The current-voltage relationship of gap junction is usually linear and sometimes asymmetric, rectifying, which provides one-direction flow. In addition to ion conductance, several lines of evidence implicate gap-junction channels in mediating the propagation of intercellular Ca²⁺ waves, leading to the synchronization of cellular function in a particular tissue. Because of the nonselectivity of gap-junctions to ions and small molecules, several diffusible second-messenger molecules are potential candidates for mediating the propagation of intercellular Ca²⁺ waves via gap junctions, including Ca²⁺ and IP₃.

7. ACTIVE TRANSPORTERS

The primary purpose of active transporters is to generate and maintain the transmembrane ionic concentration gradients, which in turns provide the driving forces for ion channels to generate electrical signals. In contrast with channels, which can conduct thousands of ions through the pore each millisecond, ion translocation by active transporters requires ion binding and unbinding for transporter, a process that requires several milliseconds. Also, transporters need energy for ion movement against their concentration gradients. The energy for such uphill transport is provided by two sources. One group of transporters requires ATP for its action and they are called *ATPase pumps*. The most important pump is Na-K pump, which maintains the high Na⁺ and K⁺ gradients across the plasma membrane. Plasma membrane Ca²⁺ pump also requires ATP to remove intracellular Ca²⁺. Such pumps are also called *primary active transporters*. Other transporters are responsible for the electrochemical gradients, such as Cl⁻, Ca²⁺, and H⁺. They are classified as *secondary active transporters*, as the driving force for catalysis is not coupled directly to the hydrolysis of ATP but is derived from the electrochemical gradients established for one of the solutes (usually Na⁺) that drives countertransport of the other. Because at least two different ions are involved in such transaction, the secondary transporters are also called *ion exchangers*. An *antiporter* is an integral membrane protein that is involved in active transport of two or more different ions across the plasma membrane in opposite directions, whereas a *symporter* is an integral membrane protein that is involved in active transport of two or more different ions across the plasma membrane in the same direction.

7.1. Na-K-ATPase Has Multiple Functions

The *Na-K ATPase*, also known as *Na pump*, is a membrane-bound protein that establishes and maintains the high internal K⁺ and low internal Na⁺ concentrations typical of most animal cells. The activity of this transporter is estimated to account for 20% to 40% of the brain energy consumption. By using the energy from the hydrolysis of one molecule of ATP, this pump transports Na⁺ out of the cell in exchange for K⁺ transported into the cell. The pump with bound ATP binds three intracellular Na⁺ ions. Then ATP is hydrolyzed, leading to phosphorylation of the pump at a highly conserved aspartate residue, the subsequent release of ADP, and a conformational change in the pump exposing the

Na⁺ ions to the outside. The phosphorylated form of the pump has a low affinity for Na⁺ ions, so they are released. The pump then binds two extracellular K⁺ ions, causing the dephosphorylation of the pump and reverting it to its previous conformational state, and exposing the K⁺ ions to the inside of the cell. As the unphosphorylated form of the pump has a higher affinity for Na⁺ ions than for K⁺ ions, the two bound K⁺ ions are released. Thus, there is a net loss of one positive charged ion from inside of the cell during each round of pumping, indicating that the pump generates an electrical current that can hyperpolarize the membrane potential. Such transport is termed *electrogenic*. The electrochemical gradient the Na pump generates is not only critical in maintaining the resting membrane potential and excitability of cells but also for maintaining the osmotic balance of the cell. In the kidney, the Na pump plays a major role in driving the reabsorption of Na⁺ and water. Finally, the Na⁺ gradient provides the energy for the Na-coupled transporters.

Na pump is an oligomer composed of two major polypeptides, the α and β subunits. The α subunit spans the membrane 10 times, whereas the β subunits spans the membrane once. The α subunit contains the binding sites for cations, ATP, and ouabain, an inhibitor of this pump, and the β subunit is essential for the normal activity of the enzyme (Fig. 28A). A third protein, termed the γ subunit, has also been identified in purified preparation of the enzyme. There are four subtypes of α subunits, $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 4$, and three β isoforms, $\beta 1$, $\beta 2$, and $\beta 3$. The distribution of these isoforms is tissue- and developmental-specific. Alternative splicing of mRNAs for these subunits further contributes to the generation of different isozymes. The isozymes exhibit different sensitivity to ouabain.

7.2. Na-Ca Exchanger and Ca-ATPase Control Intracellular Calcium

The intracellular Ca²⁺ concentration is controlled by two major pathways, *sodium-calcium exchanger* (often denoted *Na⁺/Ca²⁺ exchanger* [NCX] or *exchange protein*) and *Ca²⁺-ATPases* or *Ca²⁺ pumps*. NCX uses the energy that is stored in the electrochemical gradient of Na⁺ by allowing this ion to flow down its gradient across the plasma membrane in exchange for the countertransport of Ca²⁺. The stoichiometry of NCX is generally accepted to be removal of a single calcium ion in exchange for the import of three sodium ions. In addition to this transport mode, it has been shown that ion flux ratio can vary from 1:1 to a maximum of 4:1, depending on the

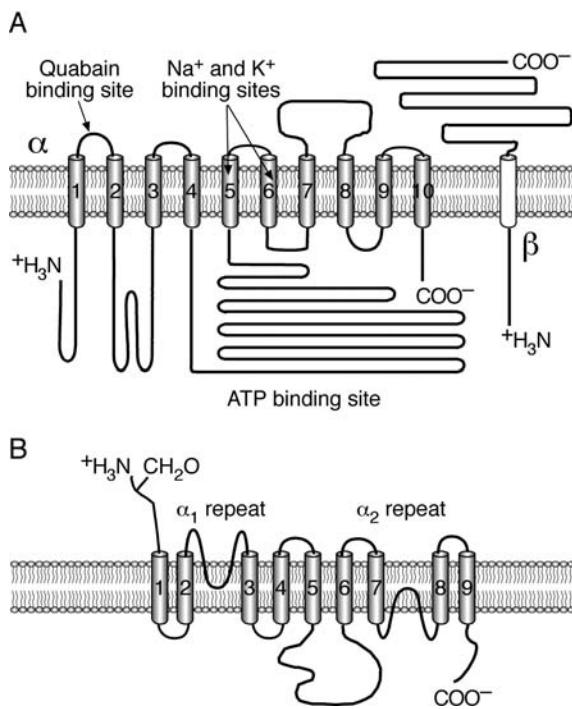


Fig. 28. Transmembrane organization of sodium pump and sodium-calcium exchanger. **(A)** Topological model of the Na-K-ATPases. Arrows indicate transmembrane domains and loops that are important for receptor functions. (Derived from G. Blanko and R.W. Mercer, Am J Physiol 1988; 255:F633.) **(B)** Topological organization of the Na/Ca²⁺ exchanger. (Derived from L. Annunziato, G. Pignataro, and G.F. Di Renzo, Pharmacol Rev 2004; 56:633.)

intracellular Na⁺ and Ca²⁺ concentrations. When intracellular Na⁺ concentrations are elevated, the NCX exchanger mediates the extrusion of Na⁺ and influx of Ca²⁺, and this model of operation is defined as “reverse way.” The NCX may operate in both forward and reverse directions simultaneously in different areas of the cell, depending on the combined effects of Na⁺ and Ca²⁺ gradients. The NCX does not bind very tightly to Ca²⁺ (has a low affinity), but it can transport the ions rapidly (has a high capacity), transporting up to 5000 Ca²⁺ ions per second. Therefore, it requires large concentrations of Ca²⁺ to be effective but is useful for ridding the cell of large amounts of Ca²⁺ in a short time, as is needed in a neuron after an action potential.

Different isoforms of NCX exist, named NCX1, NCX2, and NCX3. NCX1 is composed of 938 amino acids and has nine TM segments, with N-terminus located extracellularly and C-terminus located intracellularly (Fig. 28B). The first five TM segments are separated from the last four segments through a large hydrophilic intracellular loop containing 550 amino

acids, termed the f-loop. This loop is not involved in transport of ions but represents the regulatory domain of the exchanger, including the PKA and PKC phosphorylation sites. NCX2 and NCX3 consist of 921 and 927 amino acids, respectively. NCX1 gene is expressed in numerous tissues, including brain, heart, skeletal muscle, smooth muscle, kidney, and secretory and blood cells, whereas NCX2 and NCX3 have been found exclusively in neuronal and skeletal muscle tissues. NCX1 and NCX3 give rise to several splicing variants. Another, more ubiquitous transmembrane pump that exports Ca²⁺ from the cell is the plasma membrane Ca²⁺-ATPase, which has a much higher affinity but a much lower capacity. Because this pump is capable of effectively binding to Ca²⁺ even when its concentrations are quite low, it is more appropriate for maintaining the very low concentrations of intracellular Ca²⁺ that are normally within a cell. Therefore, the activities of the NCX and the plasma membrane Ca²⁺-ATPase complement each other. There is another subtype of Ca²⁺-ATPase, located in the ER and sarcoplasmic reticulum membranes, which pumps Ca²⁺ from cytosol to the lumen of these organelles.

7.3. Several Transporters Maintain Chloride Gradient

There are three major secondarily active transport proteins involved in establishment and maintenance of neuronal chloride gradient: the *cation-chloride co-transporters* (CCCs), the Na⁺-dependent anion exchangers (NDBEs), and the *Na⁺-independent anion exchangers* (AEs). As HCO₃⁻ is a substrate for AEs and NDBEs, these two transporters are also directly involved in intracellular pH regulation (see Section 7.4). Transport of Cl⁻ mediated by CCCs is electroneutral and the energy for net transports derived from the cation gradients generated by Na pump. The CCCs proteins are glycoproteins with a relatively small intracellular N-terminus followed by 12 TM segments and a large intracellular C terminus. The CCC gene family consists of two major groups: *K⁺-Cl⁻ co-transporters* (KCCs) and *Na⁺-K⁺-Cl⁻ co-transporter* (NKCCs). The KCCs are encoded by four separate genes. KCC1 and KCC2 expression is restricted to CNS; KCC3 is expressed in central and peripheral neuronal tissues and exists in two splice forms; and KCC4 is predominately expressed in heart and kidney. Two genes encode NKCCs: NKCC1 expressed in both epithelial and nonepithelial cells, and NKCC2, which is selectively expressed in kidney. The membrane topology of NKCC1 is shown in

Fig. 29B. The third gene encodes the *atypical Na⁺-Cl⁻ co-transporter* (NCC), exclusively expressed in kidney. In addition, two novel proteins, called CCC8 and CCC9, have been identified recently.

NCCs and NKCCs mediate active chloride uptake, promoting intracellular accumulation of this ion, and this transport is driven predominately by the energy taken from the Na⁺ concentration gradient. In contrast, KCCs mediate active chloride extrusion, and K⁺ gradient provides the energy for the operation of this exchanger. The opposite effects of NKCC1 and KCC2 on intracellular chloride concentrations play a major role in developmental transition of GABA channels from excitatory in prenatal life to inhibitory in postnatal life. It appears that this transition is associated with downregulation of NKCC1 expression together with upregulation of KCC2 expression after birth. Experiments with the NKCC1 and KCC2 knock-out mice confirmed these conclusions. Specifically, basal intracellular chloride concentration was significantly reduced in the NKCC1 knockout mice, whereas KCC2 knockout mice die immediately after birth and GABA and glycine were excitatory in neurons from KCC2-null mice. In the presence of modest residual expression of the KCC2, mice survive but exhibit almost constant epilepsy.

7.4. Acid-Base Transporters

Intracellular pH in mammalian tissues is regulated through coordinated action of several acid-base transporters, including the above-mentioned NDBEs and AEs, Na⁺-H⁺ exchangers (NHEs), and Na⁺:HCO₃⁻ co-transporters (NBCs). Sodium/proton antiporters or exchangers (NHE) are ubiquitously expressed integral membrane proteins that regulate intracellular pH by removing a proton in exchange for an extracellular Na⁺. In mammals, these transporters are crucial for numerous physiologic processes, ranging from the fine control of intracellular pH and cell volume to systemic electrolyte, acid-base, and fluid volume homeostasis. NHE activity also facilitates the progression of other cellular events such as adhesion, migration, and proliferation. Eight distinct NHE genes (NHE1 to NHE8) and several pseudogenes have been identified in the human genome. The functional genes encode proteins of varying primary sequence identity (25% to 70%) but share a common predicted secondary structure comprising 12 conserved membrane-spanning segments at the N-terminus and a more divergent, cytoplasmically oriented, C-terminus (Fig. 29A). N-terminal membrane domain is involved in transport

of ions, whereas the C-terminal domain regulates the activity of exchanger and mediates cytoskeletal interactions. NHEs show considerable heterogeneity in their patterns of tissue/cell expression and membrane localization. Functional studies have revealed further differences in their kinetic properties, sensitivity to pharmacologic antagonists, and regulation by diverse hormonal and mechanical stimuli.

Recent molecular cloning experiments have identified the existence of four NBC isoforms (NBC1 to 4) and two NBC-related proteins AE4 and NCBE. All but AE4 are presumed to mediate the co-transport of Na⁺ and HCO₃⁻ under normal conditions and may be functionally altered in certain pathologic states. NBC1 is essential for bicarbonate transport across plasma membranes in both epithelial and nonepithelial cells. The direction of the NaHCO₃ movement in secretory epithelia is opposite to that in reabsorptive epithelia. In secretory epithelia (such as pancreatic duct cells), NBC is responsible for the transport of bicarbonate from blood to the cell for eventual secretion at the apical membrane. In reabsorptive epithelia (such as kidney proximal tubule cells), NBC is

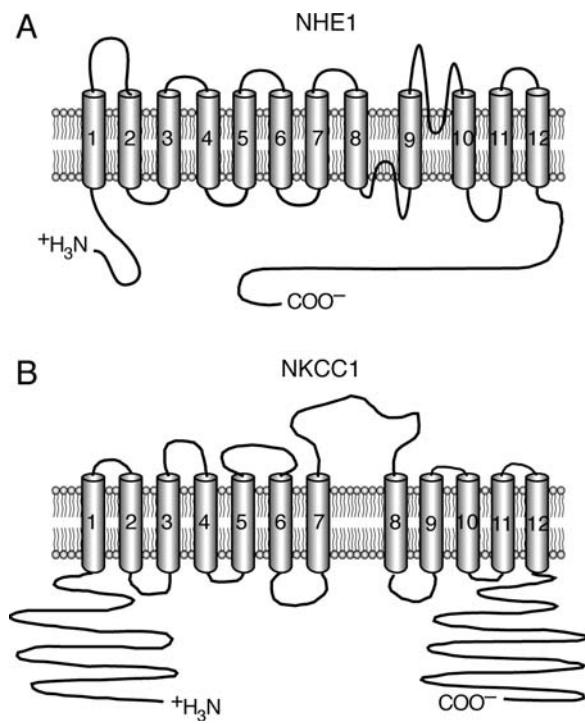


Fig. 29. Transmembrane organization of (A) the Na⁺/H⁺ exchanger and (B) the Na⁺-K⁺-Cl⁻ co-transporter. (Topological model of NHE1 and NKCC1 are derived from J. Orlowski and S. Grinstein, Eur J Physiol 2004; 447:549; and S.F. Pederson, M.E. O'Donnell, S.E. Anderson, and P.M. Cala, Am J Physiol 2005; 291:R1, respectively.)

responsible for the reabsorption of bicarbonate from cell to the blood. In nonepithelial cells, this transporter is mainly involved with cell pH regulation. NBC1 shows a limited tissue expression pattern, is electrogenic, and plays an important role in bicarbonate reabsorption in kidney proximal tubule. In addition to the kidney, NBC1 is expressed in pancreatic duct cells, is activated by CFTR, and plays an important role in HCO_3^- secretion. NBC2 and NBC3 have a wider tissue distribution than does NBC1, are electroneutral, and are involved with cell pH regulation. The NBC4 is incompletely characterized.

7.5. Other Na^+ Gradient-Driven Transporters

At least three, and up to six, Na^+ -dependent glucose transporters (SGLT1 to SGLT6) may exist. Similarly, 13 members of the family of facilitative sugar transporters (GLUT1 to GLUT12 and HMIT) are now recognized. These various transporters exhibit different substrate specificities, kinetic properties, and tissue expression profiles. Sodium-glucose transport proteins are a family of glucose transporters found in the intestinal mucosa of the small intestine (SGLT1) and the proximal tubule of the nephron (SGLT2 and SGLT1). These proteins use the energy from a downhill sodium gradient to transport glucose across the apical membrane against an uphill glucose gradient. Therefore, these co-transporters are an example of secondary active transport.

8. A CROSS-COMMUNICATION BETWEEN ELECTRICAL AND RECEPTOR-MEDIATED SIGNALING PATHWAYS

Hormones and neurotransmitters acting through their respective receptors can modulate the gating properties of voltage-gated and ligand-gated channels. This modulation is dependent on signal-transduction pathways of receptors. These include direct action of heteromeric G proteins on channels (termed *membrane-delimited pathway*) and indirect action, through intracellular messengers, including Ca^{2+} , cyclic nucleotides, nitric oxide, ATP, and PKA, PKC, and PKG (termed *intracellular messenger-dependent pathway*) (Fig. 4). The first pathway is limited to the regulation of a few channels, whereas the second pathway represents a common mechanism by which receptors can influence channel activity.

Inhibition of Ca_v channels can occur through the fast membrane-delimited pathway, in which the $\beta\gamma$ dimer of the G_i/G_o proteins is a direct intermediate between the plasma-membrane receptor and channels.

This is a time- and voltage-dependent process. As a fraction of the total Ca^{2+} channels open much more slowly and require larger depolarization to open, this produces a slowing of the activation kinetics and a reduction in the current amplitude. In contrast, several G_i/G_o -coupled receptors, including dopamine, somatostatin, and endothelin-A, activate K_{ir} channels in $\beta\gamma$ dimer-dependent manner, leading to hyperpolarization of the membrane, cessation of action-potential firing, and a decrease in intracellular Ca^{2+} concentration and hormone secretion.

The activity of Ca_v channels can also be modulated in a second messenger-dependent manner. For example, in cardiac myocytes, activation of PKA augments Ca^{2+} influx through Ca_v channels. The effects of PKA phosphorylation have been attributed to an increase in the probability of the channel being open and to an increase in the mean open time. Also, in rat and human pituitary adenoma cells, pituitary adenylate cyclase-activating polypeptide stimulates TTX-sensitive Na_v channels via an adenylate cyclase-PKA pathway to increase hormone secretion. Phosphorylation also plays an important role in the modulation of several other channels, including BK , K_{ir} , IP_3Rs , and P2XRs.

Receptors can also modulate the activity of another K^+ channel, the M-type. Inhibition of this current was first observed in response to the activation of muscarinic receptors in bullfrog sympathetic ganglion neurons, as indicated by the name M-type current. Since then, it has been demonstrated that activation of a variety of receptors suppresses this current, including GnRH, bradykinin, opioids, substance P, ATP, adrenergic, TRH, and angiotensin II receptors. Because these receptors are typically coupled to the phospholipase C pathway, initially it was believed that IP_3 -induced Ca^{2+} release accounts for this inhibition. Recent studies identified phosphatidylinositol 4,5-bisphosphate as an essential molecule in regulation of M-current. Intracellular and extracellular Ca^{2+} is also involved in control of the gating of several other channels. We have already discussed K_{Ca} and Cl_{Ca} channels. Calcium also activates a nonselective cation channel. L-type Ca^{2+} channels are inhibited in a Ca^{2+} /calmodulin-dependent manner. Calcium is a principal factor controlling RyR gating and cofactor in controlling IP_3R gating. Calcium also inhibits the conductivity of CNG channels in a dual mechanism, directly and through a calmodulin-dependent mechanism.

Several other intracellular molecules can modulate the gating of channels. As previously discussed, cyclic nucleotides are crucial in the regulation of two channels,

CNG and HCN. Thus, activation of adenylyl-cyclase pathway by G_s-coupled receptors leads to stimulation of both channels, whereas activation of G_i/G_o-coupled receptors leads to a decrease in cAMP production and a silence of these channels. Nitric oxide provides an additional mechanism for stimulation of cGMP production (through activation of soluble guanylyl cyclase). Nitric oxide itself has been implicated as a messenger in regulating CNG and BK channels. Intracellular ATP and ADP control K_{ir}6 channels.

Electrical activity and the associated Ca²⁺ influx also influence the receptor-mediated intracellular signaling. Voltage-gated Ca²⁺ influx has been shown to play an important role in sustained activation of phospholipases C and D. Spontaneous electrical activity is coupled to the activation and/or inhibition of adenylyl cyclase, nitric oxide synthase, and soluble guanylyl cyclase activities. There is also a cross-communication between plasma membrane and ER channels. For example, voltage-gated Ca²⁺ influx facilitates IP₃-mediated Ca²⁺ release during the sustained agonist stimulation. Thus, two pathways for signaling in neurons, electrical and receptor-controlled, are not independent of each other but interact to provide the synchronized control of cellular functions.

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Demyelinating Disorders

Gregory Cooper, Gerald Eichhorn and Robert Rodnitzky

Disorders of myelin can be divided into conditions in which there is destruction of myelin—the demyelinating disorders—and those in which there is an abnormality in the makeup of myelin, known as the dysmyelinating disorders. Demyelination can result from a great variety of causes, including autoimmune, toxic, metabolic, infectious, or traumatic conditions. The dysmyelinating disorders are heritable conditions resulting from inborn errors of metabolism that affect myelin. Central or peripheral nervous system myelin can be affected in these conditions, but most clinical syndromes related to disorders of myelin primarily affect the brain and spinal cord.

IMPAIRED OR BLOCKED IMPULSE CONDUCTION

Impaired or blocked impulse conduction is the cause of most clinical symptoms related to myelin disorders. Demyelination prevents saltatory conduction in normal rapidly conducting axons. Instead, impulses are propagated along the axon by continuous conduction, or in the extreme, there is total conduction block. The latter may result from the markedly prolonged refractory period in demyelinated segments, explaining why rapidly arriving, repetitive impulses are especially likely to be blocked.

Demyelination may also affect conduction by rendering axon function more susceptible to changes of the internal milieu, such as alterations of pH or temperature. The enhanced effect of temperature change on the function of demyelinated central nervous system (CNS) pathways, or *Uhthoff's phenomenon*, is well-known and can be the cause of prominent and precipitous neurologic symptoms after temperature

elevation in patients with multiple sclerosis. Persons who experience this phenomenon notice the onset of new symptoms, such as monocular blindness, extremity weakness, or incoordination, whenever they develop a mildly elevated body temperature. With more profound elevation of body temperature, such as that occurring during a warm bath or as a result of a fever, neurologic dysfunction can be still more severe. Usually, when body temperature reverts to normal, neurologic function returns to baseline. Uhthoff's phenomenon is based on the principle that conduction block supervenes in demyelinated fibers when they reach a critical threshold temperature. This threshold temperature may be just above normal body temperature, explaining why such symptoms may develop after only a slight temperature elevation, such as that seen as part of normal diurnal variation.

DISEASES THAT AFFECT MYELIN

Many different processes can be involved in the pathogenesis of myelin disorders. Adrenoleukodystrophy is an example of a metabolic disorder of myelin. It is an X-linked peroxisomal disorder in which there is impaired oxidation of very-long-chain fatty acids, with resultant abnormalities of myelin.

Progressive multifocal leukoencephalopathy is an infectious condition resulting in demyelination. It is caused by an opportunistic viral infection of oligodendroglial cells in immunosuppressed patients, such as those with acquired immunodeficiency syndrome. Oligodendroglial death leads to widespread demyelination, especially in the posterior portions of the cerebral hemispheres. The resultant severe neurologic dysfunction is ultimately fatal.

Radiation is an example of a form of trauma that can lead to demyelination. For this reason, patients undergoing radiation therapy in which the brain or spinal cord is in the field of treatment may later develop neurologic symptoms. Toxic agents such as cancer chemotherapy drugs can result in demyelination, especially when instilled directly into the cerebrospinal fluid (CSF).

Autoimmune mechanisms can also result in demyelination. This process is believed to be important in the pathogenesis of one of the most common demyelinating disorders, multiple sclerosis (MS).

MS HAS A DISTINCT AGE, GENDER, RACE, AND GEOGRAPHIC PROFILE

MS is a relatively common neurologic disorder. The prevalence of MS in the United States and Northern Europe is approximately 100 per 100,000, and almost 300,000 Americans are afflicted with this condition. Women are affected 1.5 times more often than men. The age distribution of MS is distinct in that onset before the age of 15 years or after the age of 45 years is unusual. The Caucasian population, especially persons of northern European ancestry, has a much higher incidence of MS than do those of Asian or African descent.

Within the United States and to a certain degree throughout the world, there is a distinct geographic distribution of MS, with temperate zones having the highest incidence. In the United States, this distribution results in a strikingly higher prevalence of MS in the northern-most states than in the southern-most states. The precise cause of these geographic variations remains unclear, but some migration studies have suggested a role for environmental factors, such as a viral exposure during childhood, as emigration from a high to a low prevalence zone before adolescence appears to reduce the risk of developing MS.

MS IS CHARACTERIZED BY THE PRESENCE OF NUMEROUS DISCRETE AREAS OF DEMYELINATION THROUGHOUT THE BRAIN AND SPINAL CORD

In MS, multiple plaques of demyelination are found in the CNS. Within these areas, there is evidence of inflammation, proliferation of glial tissue, and severe destruction of myelin. In these same areas, axons may remain largely intact. Minimal remyelination of fibers

may occur in some plaques, resulting in areas with scant myelin known as “shadow plaques.” The pathogenesis of demyelination within these plaques is not fully understood, but it is generally agreed that it involves an immune response mediated by T lymphocytes that recognize myelin components of the CNS. The pathology of MS may change during the course of the disease with less prevalent inflammatory changes and prominence of neurodegenerative mechanisms leading to neuroaxonal atrophy. Plaques may occur anywhere in the CNS, but they have a predilection for certain parts of the brain and spinal cord, forming the anatomic basis for the most common clinical symptoms of MS. The most common regions of involvement are the optic nerves, the cerebellum, the periventricular white matter of the cerebral hemispheres, the white matter of the spinal cord, the root-entry zones of spinal or cranial nerves, and the brain stem, especially the pons.

THE SYMPTOMS OF MS REMIT AND REAPPEAR IN CHARACTERISTIC FASHION

MS is marked by a wide range of disparate neurologic symptoms that can occur in a single affected person and, in the most common form, a tendency for symptoms to appear, spontaneously improve, and then reappear or be replaced by new symptoms over time. This relapsing-remitting course may give way to a more gradually progressive course over time. This tendency toward spontaneous remittance of neurologic symptoms is highly characteristic of MS. Typically, serious neurologic symptoms appear and disappear over a period ranging from weeks to several months, independent of medical therapy. The variety of symptoms in a particular patient reflects the widespread dissemination of MS plaques throughout the brain and spinal cord. In a typical MS patient, it is not usually possible to attribute all neurologic symptoms to a single anatomic locus of abnormality. Rather, multiple areas of demyelination must be invoked. Therefore, patients with MS experience symptoms reflecting episodes of demyelination within the CNS that is separated both in time and space.

Certain neurologic symptoms are much more likely to occur in MS, reflecting the predilection for certain anatomic sites of involvement alluded to previously. These include monocular visual loss (reflecting involvement of the optic nerve), limb incoordination (cerebellum), double vision (brain stem, or medial longitudinal fasciculus), and paraparesis (spinal

cord). Other common symptoms include vertigo, extremity numbness, slurred speech, and bladder dysfunction. *Lhermitte's sign* is a frequent finding in MS. It consists of a shock-like sensation whenever the neck is flexed forward. This phenomenon is caused by the heightened sensitivity of demyelinated dorsal column axons to the mechanical stimulation of stretch when the spinal cord is flexed by the offending neck motion.

THE DIAGNOSIS OF MS CAN BE AIDED BY IMAGING STUDIES AND LABORATORY TESTS

Although the unique clinical pattern of MS often allows the diagnosis to be made with confidence, several diagnostic tests are extremely useful in equivocal cases. Magnetic resonance imaging (MRI) of the brain and spinal cord is a sensitive means of demonstrating multiple areas of demyelination, many of which may prove to be clinically silent. In addition, magnetic resonance imaging can determine whether a given area of demyelination is quiescent or in an active state of evolution. Active plaques tend to enhance after the administration of gadolinium contrast, indicating a disruption of the blood-brain barrier.

Examination of the cerebrospinal fluid (CSF) is used to screen for CNS immunoglobulin (IgG) abnormalities, which are common in MS. The rate of IgG synthesis within the CNS, which is typically elevated in MS, as well as the makeup of IgG within the CSF can be evaluated. When normal CSF is subjected to immunoelectrophoresis, the IgG are diffusely represented at the cathodal region. In MS, the electrophoretic pattern of the cerebrospinal fluid demonstrates one or more discrete bands of IgG not seen in a paired serum sample. These bands are known as oligoclonal bands. These are presumed to be specific antibodies, but the antigen(s) against which they are directed have not yet been identified. Testing for the presence of oligoclonal IgG bands is extremely useful in diagnosing MS because as many as 95% of individuals with proven MS have been found to exhibit this abnormality. Another useful CSF study involves myelin basic protein, a breakdown product of myelin. Because it can be detected in the CSF in the presence of active CNS demyelination, it can be considered an indicator of disease activity.

Electrophysiologic studies such as visual and somatosensory evoked potentials are used to detect subtle physiologic abnormalities in MS that are not yet

sufficiently advanced to produce clinical symptoms. These tests measure the speed and completeness of conduction in sensory pathways. The technique involves delivery of a sensory stimulus such as a flash of light, measuring the time required for an evoked response to be reflected in the cortex and recorded by a scalp electrode. In patients with a history of overt sensory symptoms, slowed conduction can often still be demonstrated in the appropriate sensory pathway even after these symptoms remit.

IMMUNOSUPPRESSION IS THE MOST COMMON FORM OF THERAPY USED IN MS

The propensity for MS symptoms to remit spontaneously makes the scientific evaluation of potential therapies difficult. In the past, poorly designed or uncontrolled studies have led to a multitude of false claims of therapeutic efficacy for a variety of therapies, some of which were quite unorthodox. However, a number of agents now exist for the treatment of MS, which presumably act through an immunomodulatory mechanism. The first agents to be approved by the FDA were interferon-beta-1b (Betaseron) and interferon-beta-1a (Avonex). Putative mechanisms for these medications include inhibition of autoreactive T cells, inhibition of MHC class II expression, metalloproteinase inhibition, altered expression of cell-associated adhesion molecules, and induction of immunosuppressive cytokines and inhibition of proinflammatory cytokines. A third agent, glatiramer acetate (Copaxone), is a polypeptide containing random arrangements of four basic amino acids, believed to mimic myelin basic protein. This agent is believed to act by inducing myelin-specific suppressor T cells and inhibiting myelin-specific effector T cells. Natalizumab (Tysabri), a humanized monoclonal antibody that binds to the $\alpha 4\beta 1$ integrins of leukocytes, blocks attachment to cerebral endothelial cells, thus reducing inflammation at the blood-brain barrier. All of these agents are used in an attempt to slow progression of the disease or to reduce the frequency of relapses. In clinical trials of natalizumab, a small number of patients developed progressive multifocal leukoencephalopathy (PML), illustrating the potentially serious side effect of infection related to immunosuppression.

For the treatment of acute relapses, the most commonly used treatments are the corticosteroid drugs, prednisone or methylprednisolone. These agents are usually administered at high doses and are believed to help reduce inflammation and restore the

blood-brain barrier, thereby shortening the course of relapse and speeding recovery.

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Henrique von Gersdorff

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OVERVIEW

The nervous system is composed of specialized cellular circuits that allow an animal to perform tasks essential for survival. Neurons are organized to form these circuits, and they transmit electrical and chemical signals among themselves to process sensory input, initiate behavioral responses, and regulate an animal's internal physiology. The critical link between neurons that permits communication and establishes the foundation for neuronal circuitry is called the *synapse*, and this chapter will discuss fundamental synaptic properties.

Synapses are sites of close cellular contact where fast, highly localized transmission of chemical and electrical signals can occur. The human brain has approximately 10^{11} neurons that form about 10^{15} synapses. By comparison, the simple nematode worm *C. elegans* has exactly 320 neurons with only about 7600 synapses. The capacity of the human brain to form such an astronomical number of synapses has surely contributed to the success of our species and its vast repertoire of behaviors. In order to understand how synapses confer such complexity of neuronal circuitry, it is important to explore the details of information transfer at the synapse.

The process of communication between neurons, termed *synaptic transmission*, is also key to developing better medical treatments of neurologic conditions for several reasons. The causes of several mental disorders and neuromuscular diseases can be traced to dysfunctional synapses. Synapses are also the locus of action for several neurotoxins and psychoactive drugs (some of which can cause debilitating and life-long addictions). Finally, determining how synapses transmit signals and how neuronal circuits are remodeled and modulated at the synaptic level will eventually allow us to understand the basis of neuronal learning and memory.

Synapses vary widely in shape, size, and functional capability. Presumably, such architectural and functional diversities are tailored for the specialized information transfer and processing needs of individual neurons and circuits. For example, many synapses function as high-fidelity relay stations. The connection between motor neurons and muscle fibers (termed the *neuromuscular junction*), the giant synapses in the mammalian and avian auditory systems involved in sound localization, and the squid giant synapse, which allows a rapid escape behavior, are all examples of high-fidelity relays. These are synapses where reliability is at a premium, and the synaptic architecture is designed as a fail-safe mechanism for information transfer. Other synapses, such as the bouton-type

synapses of the cortex and hippocampus, often fail to transmit signals and are thus considered comparatively unreliable. These bouton synapses, however, have the capacity to become more fail-safe with repetitive use. This type of change in synaptic strength is an example of *plasticity* and is thought to underlie the long-lasting storage of information acquired through repetitive use of an associated neuronal circuit. In other words, the specific strengthening of a particular set of synaptic connections may form the basis for some types of learning and memory. Equally important may be the weakening of synaptic connections, a process that could either cause the loss of certain synaptic memory or endow the freedom for retasking a particular neuronal circuit. Thus, synapses must be considered as highly dynamic and plastic structures that can adapt their output to match the demands imposed by their current information processing needs. In this sense, the brain is not “hard wired” and differs fundamentally from an electronic computer.

One consequence of evolution that unifies biology and medicine is the cross-species commonality of underlying mechanisms for critical physiologic processes such as synaptic transmission. From genomes to protein structure and function, common molecular motifs are homologously conserved across phylogenetically distant species. Neurobiologists have thus been able to use non-human animal models as a means to study and understand synaptic function. Because of an unparalleled ease of access, much of the pioneering work in the field of synaptic transmission comes from studies of the frog neuromuscular junction and squid giant synapse. In addition, relatively new preparations such as the giant bipolar cell synapse from goldfish retina and calyx of Held synapse in the mammalian brain stem have shed much new light on our understanding of synaptic function. These and many other preparations have yielded a wealth of information about synapses and revealed several general principles that apply directly to synaptic transmission in the human brain. It is these general principles of synaptic transmission that will be reviewed in this chapter.

1. PROPERTIES OF CHEMICAL AND ELECTRICAL SYNAPSES

Neurons communicate using morphologically and functionally specialized sites of close contact called synapses. Synaptic transmission can be electrical or chemical, though the vast majority of synapses in the mammalian brain are chemical. At *chemical synapses*, molecules of neurotransmitter are released from a

presynaptic terminal into a narrow extracellular gap (about 20 to 50 nm) called the *synaptic cleft*. The transmitter molecules then diffuse and bind to recognition sites on target receptors at the plasma membrane of a postsynaptic neuron. This type of synaptic transmission is fast, site-specific, and highly plastic.

A different type of synaptic transmission occurs at *electrical synapses*. Here, proteins form *gap-junctions*, which create a conductive pore between two neurons. This pore is a ionotropic transmembrane channel composed of connexin proteins on the plasma membrane of each neuron that allows ions and small molecules (e.g., cAMP, ATP, Ca^{2+} , IP_3) to cross between cells. The cytoplasm of two neurons connected by a gap junction is thus physically continuous, and the resulting low-resistance channel allows *electrical coupling*. Transmission at electrical synapses is *bidirectional*, although some gap junctions may transmit better in one direction (i.e., they show *rectification*).

Although electrical coupling limits the variety of signaling between neurons (electrical activity in one neuron is identically passed to its connected partner), it allows even faster communication than does chemical signaling and can synchronize the activity of a group of cells that must work in concert. For example, every neighboring cell in the heart is connected via gap junctions, and the resultant electrical coupling allows the tissue-wide coordination of cardiac contractions.

Gap junctions are not, however, static structures. Many tissues contain gap junctions during development, which are then lost as the nervous system matures. In addition, gap junction conductances can be modulated by phosphorylation and/or neurotransmitters in order to alter the dynamic state of entire neuronal circuits. In the retina, for example, circadian changes in dopamine levels modulate the opening of gap junctions and allow retinal circuitry to adapt its light sensitivity from day to night.

By contrast, chemical synapses are far more complex than their electrical counterparts. Chemical synapses depend on an elaborate cascade of protein-protein and lipid-protein interactions that have only recently been explored at the molecular level. Some of the differences between electrical and chemical synapses are listed in Figure 1. Chemical synapses occur between axon endings (*presynaptic terminals*) that contain neurotransmitter-filled *synaptic vesicles* and postsynaptic neurons with clusters of neurotransmitter receptors. These two elements are separated by the synaptic cleft. (See Fig. 2 for an electron microscope image of a conventional brain synapse and Fig. 3 for a schematic

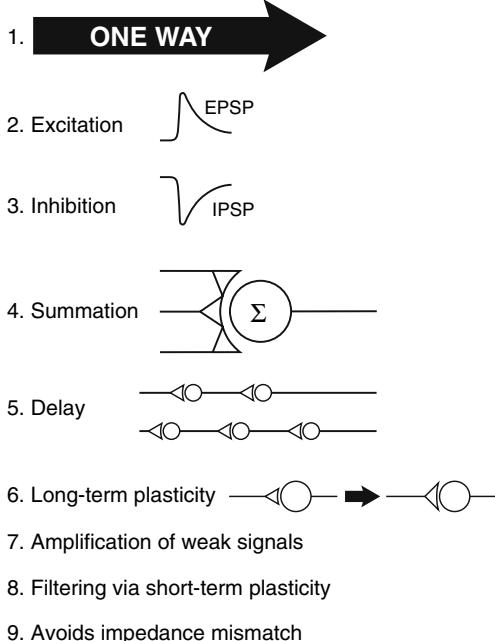
Advantages of Chemical Synapses

Fig. 1. The multiple advantages of chemical synapses. (1) Chemical synapses are mostly unidirectional and transmit from presynaptic to postsynaptic neurons. Information is thus relayed sequentially to different cells in a neural circuit. (2) Synapses can produce an excitatory postsynaptic potential (EPSP) causing the postsynaptic neuron to fire APs. (3) Synapses can also produce an inhibitory postsynaptic potential (IPSP) suppressing postsynaptic firing of APs. (4) Several small synaptic potentials can be summed by the postsynaptic cell before it fires an AP. This allows the neuron to integrate information from several different sources. (5) Chemical synapses introduce a short synaptic delay in transmission, and this can be used for calculating the timing of sensory inputs. In the example shown, information can be routed via a disynaptic or a trisynaptic pathway. (6) Synaptic strength or efficacy is plastic and can undergo changes on a long timescale (hours or days). Synaptic morphology and functional properties can thus change with experience. This is indicated by the larger synaptic connection. (7) Synapses can amplify a weak presynaptic signal. (8) Synaptic strength or efficacy is also plastic on a short timescale (milliseconds to seconds). This short-term synaptic plasticity can cause synaptic depression or fatigue if the synapse is stimulated at high frequencies. Thus, high-frequency stimulation may be filtered and not transmitted as effectively as low-frequency stimulation. (9) Synaptic transmission avoids impedance mismatch problems that may occur at electrical synapses between neurons of different sizes. (Modified from Gardner, D. Synaptic transmission. In: *Neuroscience in Medicine*, edited by Conn, M.P. J.B. Lippincott, Philadelphia, 1995).

diagram of the main elements in a synapse.) Chemical synapses are therefore polarized and primarily mediate synaptic transmission from the presynaptic terminal to the postsynaptic neuron.

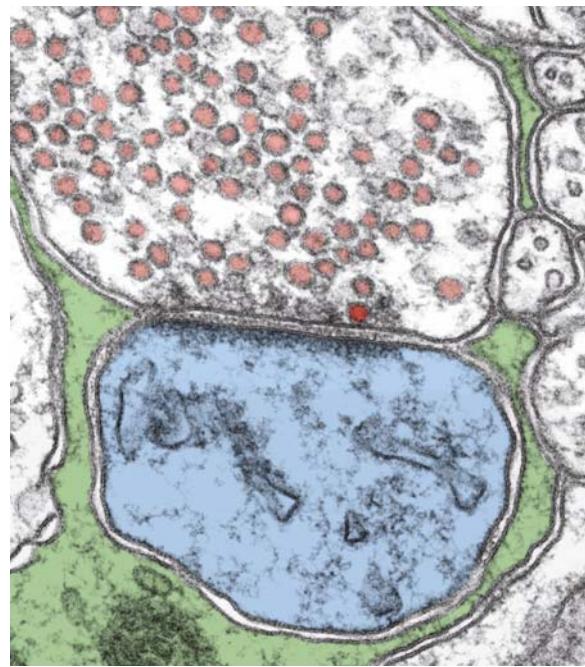


Fig. 2. An electron micrograph of a CNS synapse. This example of a synaptic bouton-type synapse is located in the “molecular layer” of rat cerebellum. A single *en passant* bouton of the parallel fibers synapses onto a single Purkinje cell spine. Note the multiple synaptic vesicles in the presynaptic bouton terminal. Several vesicles seem to be linked by thin filaments in the cytoplasm. On average, the vesicles have a diameter of about 40 nm. One synaptic vesicle is clearly docked to the presynaptic membrane. Note also the narrow synaptic cleft, which contains a “fuzzy” set of electron-dense material (this probably includes cell adhesion proteins that span the cleft). The opposing postsynaptic membrane in the postsynaptic spine has an electron-dense postsynaptic density (PSD), where glutamate receptors and modulatory proteins are located. A thin glial process wraps itself around the synaptic cleft and postsynaptic spine and also partially around the presynaptic bouton-type terminal. (Electron micrograph courtesy of Constantino Sotelo, Instituto de Neurociencias de Alicante, Spain) (see Color Plate 1, following p. 378).

Unlike electrical synapses, the synaptic cleft separating presynaptic and postsynaptic membranes does not permit any direct electrical coupling between neurons (or any degree of cytoplasmic mixing). The synaptic cleft is spanned by several different kinds of *adhesion molecules* (e.g., cadherins, immunoglobulin cell adhesion molecules, neurexins, neurogligins, integrins, etc.) that provide mechanical stability and align presynaptic vesicle fusion sites (*active zones*) opposite to clusters of postsynaptic neurotransmitter receptors. A hallmark of the active zone that has been revealed by electron microscopy is a set of *docked vesicles* situated close to the plasma membrane (from 2 to 10 vesicles per active zone ideally positioned for

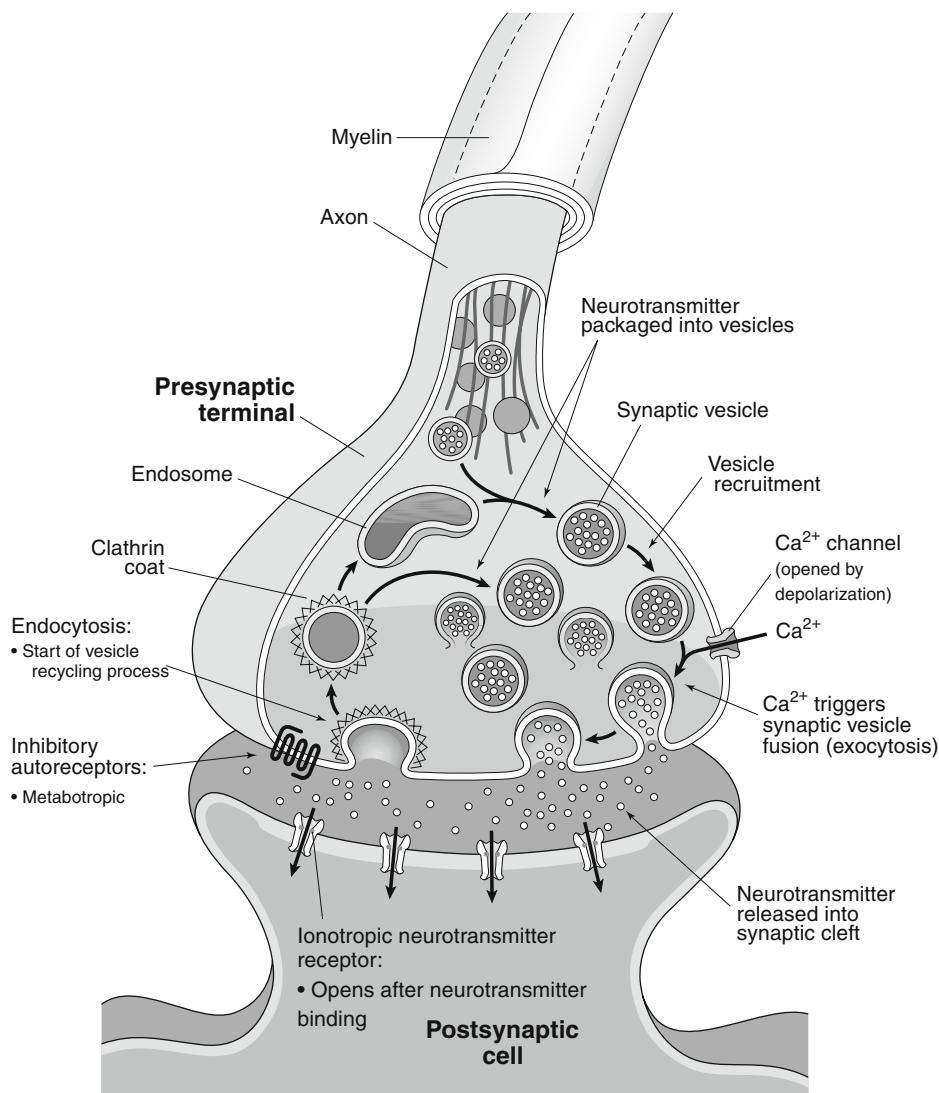


Fig. 3. Chemical synapses and synaptic vesicle recycling. Schematic diagram of the main events involved in chemical synaptic transmission at a typical bouton-type synapse. The presynaptic terminal (or bouton) is filled with neurotransmitter containing synaptic vesicles. Some vesicles are in the cytosol, constituting a reserve pool of vesicles, and some are docked at the presynaptic membrane, constituting a readily releasable pool of vesicles. Reserve vesicles can be recruited to the docked pool. A presynaptic AP depolarizes the nerve terminal and opens Ca^{2+} channels located near the docked pool of vesicles. Ca^{2+} ions trigger synaptic vesicle fusion or exocytosis. Neurotransmitter is thus released into the synaptic cleft where it binds to postsynaptic ionotropic receptors. This binding of neurotransmitter causes ion channels to open, depolarizing or hyperpolarizing the postsynaptic cell. Neurotransmitter can also bind to metabotropic receptors on the presynaptic membrane, and these can inhibit further release. Synaptic vesicle membrane that has fused with the presynaptic membrane is retrieved by endocytosis. One common form of endocytosis is clathrin-mediated endocytosis, which forms clathrin coats on endocytosed vesicles. Retrieved vesicles are then recycled via fusion to endosomes or directly back to the reserve vesicle pool where they are refilled with neurotransmitter. (Modified from Augustine, G.A. Synaptic transmission. In: *Neuroscience*, edited by Purves et al, 2001.)

immediate release). A second set of synaptic vesicles is commonly found in reserve further from the active zone, and both vesicle clusters are often thought of as discrete pools with functional differences in terms of chemical signaling. In direct apposition to the active zone, the postsynaptic membrane contains an electron-dense area called the *postsynaptic density*

(PSD). The PSD holds receptors for neurotransmitters, cytoskeletal and scaffolding proteins, and many enzymes localized to trigger signaling cascades.

Chemical transmission is initiated when an action potential (AP) invades the presynaptic terminal. The resulting membrane depolarization opens voltage-gated Ca^{2+} selective ion channels, and Ca^{2+} enters

the presynaptic neuron. This Ca^{2+} is the trigger for *exocytosis*, the process by which docked, neurotransmitter-filled synaptic vesicles fuse with the presynaptic membrane to release their contents into the synaptic cleft. The neurotransmitter is then free to diffuse across the synaptic cleft and bind to target receptors on the postsynaptic plasma membrane. These receptors are termed *ligand-gated*, and many of them are ion-selective channels that open in response to neurotransmitter binding. When these ion-selective channels open, extracellular ions flow into the postsynaptic neuron to produce either an *excitatory* or *inhibitory postsynaptic potential* (EPSP or IPSP). The type of postsynaptic potential depends largely on the particular neurotransmitter released from the presynaptic neuron and the specific receptors expressed on the postsynaptic membrane. EPSPs transiently shift the membrane potential toward more positive values, or depolarize the membrane, whereas IPSPs generally hyperpolarize the membrane. Unlike electrical synapses, chemical synapses can either maintain or invert the sign of a presynaptic signal by transforming a presynaptic excitation to a postsynaptic excitation or inhibition.

Presynaptic terminals are often less than a micrometer in diameter and frequently release only a few synaptic vesicles per AP. The effect of a small quantity of neurotransmitter on the postsynaptic membrane (the EPSP or IPSP) may therefore be insufficient for triggering a postsynaptic AP. The postsynaptic neuron, though, can be studded with up to several thousand presynaptic terminals (or boutons), and simultaneous EPSPs and IPSPs are then integrated by the postsynaptic neuron. This process of *summation* allows the postsynaptic neuron to collect input from a variety of synapses before firing its own AP. Summation of multiple inputs, along with strengthening or weakening of particular synapses, allows the brain a vast computational capacity that would be otherwise impossible with more limited and static circuitry.

Because chemical synaptic transmission is such an intricate, multistep process, there is an inherent time lag, or *synaptic delay*, that occurs between a presynaptic depolarization and a postsynaptic response. This delay, which varies between 0.1 and 0.5 ms, depends on the architecture of a particular synapse. Together with other timing cues, such as those introduced by axons of differing length, some neurons are capable of comparing sensory inputs from organ pairs such as the eyes or ears. For example, certain neurons in the auditory brain stem localize sound by comparing inputs from two different synapses

carrying signals from each ear. These neurons are *coincidence detectors* for signals arriving from each ear, and the auditory neural circuitry is precisely constructed to accommodate delays introduced by axons of differing length across several synapses.

Synapses are highly dynamic connections that can undergo both short-term and long-term changes in their morphology and transmission strength. A brief burst (or tetanus) of neural stimulation can transiently increase or decrease the amplitude of EPSPs or IPSPs. These ubiquitous phenomena are called *short-term facilitation* (increased postsynaptic potentials) or *short-term depression* (or synaptic fatigue). Synapses usually recover from short-term facilitation or depression within a few seconds. On the other hand, prolonged high-frequency stimulation of synaptic pairs can sometimes cause a *long-term potentiation* of EPSPs that can last for hours or even days. Conversely, prolonged lower-frequency stimulation of the same synapse pair may induce *long-term depression* of EPSPs. Two different synaptic inputs can therefore associate to produce what may be a simple cellular underpin for learning and memory.

Short-term depression may also act as a *frequency-selective filter*. During a tetanus, short-term depression will become increasingly potent as the stimulation frequency increases. Stimulation beyond a certain frequency is therefore filtered out at the synapse level, effectively changing the operational range of an associated neural circuit. Frequency-dependent filters are quite useful in electronics (e.g., to reduce noise and select for particular frequencies), and presumably neural circuits also make use of this synaptic property in analogous ways.

Chemical synapses also have an advantage over electrical synapses in dealing with the problem of *impedance mismatch*. When a small presynaptic cell, which has a proportionally small membrane capacitance, synapses with a larger postsynaptic cell, the smaller cell must be able to evoke a postsynaptic current of sufficient size and speed to bring the larger cell to AP threshold. If the two cells are connected via gap junctions, the smaller cell would not be able to effectively charge the membrane capacitance of the larger cell via the electronic spread of its membrane potential. Chemical synapses, however, avoid this problem by using vesicles filled with neurotransmitter that can be released several at a time. Each vesicle contains many thousand molecules of neurotransmitter, which in turn open many thousand postsynaptic ionotropic receptors. A weak presynaptic signal may therefore be amplified chemically to produce a

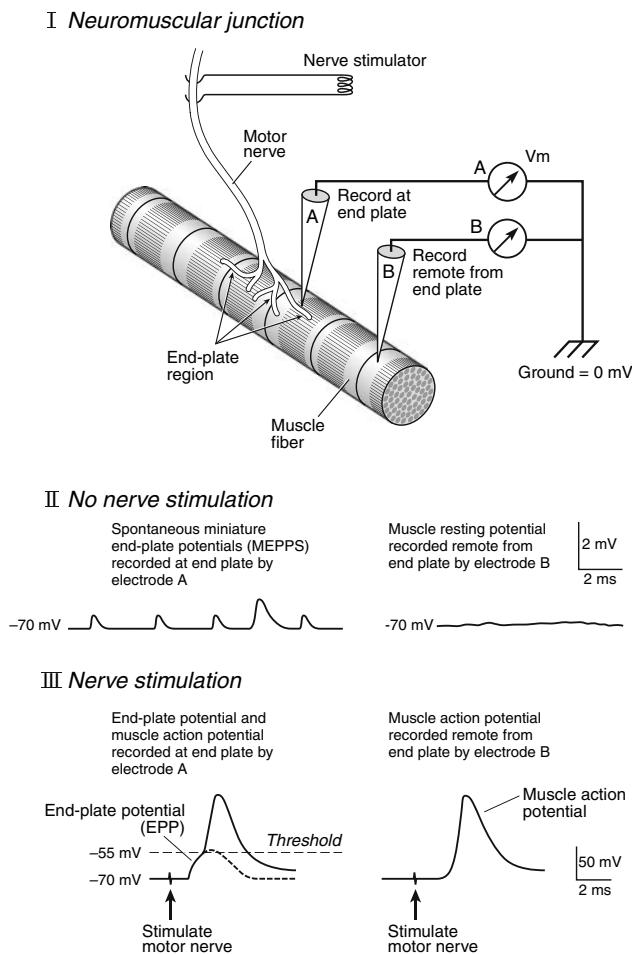


Fig. 4. The neuromuscular junction. **(I)** Schematic diagram of electrophysiologic recordings from the frog neuromuscular junction. Electrical stimulation of the motor nerve causes APs to invade the nerve terminal where they elicit transmitter release. An intracellular electrode with tip placed inside the muscle and near to the end-plate region (electrode A) can record changes in membrane voltage V_m relative to the ground potential, which is set to 0 mV. **(II)** With no nerve stimulation, small and spontaneous miniature end-plate potentials (MEPPs) are recorded by electrode A, but not by the more distantly placed electrode B, which records only the resting membrane potential of -70 mV. MEPPs are caused by the spontaneous fusion or exocytosis of single synaptic vesicles. **(III)** Upon nerve stimulation, a large end-plate potential (EPP) is observed in the motor nerve. The EPP depolarizes the nerve above the threshold for triggering a muscle AP. Electrode B also records an AP, as APs actively propagate down the muscle fiber.

comparatively larger response in the postsynaptic neuron. Such *amplification* is of particular importance at the neuromuscular junction, where the postsynaptic cell is a large muscle fiber (Fig. 4).

One challenge for chemical synaptic transmission involves rapid clearance of neurotransmitter molecules

from the cleft. In order to maintain the ability for rapid and discrete signaling, it is important that neurotransmitter does not linger in the vicinity of postsynaptic receptors causing them to remain active for prolonged periods. Though simple diffusion plays a large role in removing neurotransmitter molecules from the synaptic cleft, complete removal requires specialized enzymes. In most cases, *transporters* accomplish the task of neurotransmitter removal. Transporters are enzymes localized on the plasma membrane of neurons and glial cells, which use existing electrochemical gradients to shuttle molecules of neurotransmitter back into the cell. Pharmacologically, transporters are the locus of action for several drugs, both addictive and therapeutic. Cocaine is a specific blocker of the dopamine transporter, and the antidepressant drug Prozac (Fluoxetine hydrochloride) inhibits the serotonin transporter. At the neuromuscular junction, a different tactic is used for clearing neurotransmitter. Here, an enzyme called *acetylcholinesterase* degrades the transmitter acetylcholine in the synaptic cleft before reuptake. This enzyme is critical, and inhibiting it leads to rapid and profound paralysis. Acetylcholinesterase is the target for some insecticides, the nerve gas sarin, and the crippling autoimmune disorder myasthenia gravis.

Finally, we point out that neurotransmitters released at chemical synapses may also bind *metabotropic receptors* located on both the presynaptic and postsynaptic membranes. Unlike ligand-gated ionotropic receptors, metabotropic receptors have a higher affinity for their ligand and do not directly gate an ion channel. When located on the presynaptic terminal inside or near the synaptic cleft, they are known as *autoreceptors* because neurotransmitter released from the same cell feeds back to affect presynaptic function. Metabotropic receptors interact with bound G proteins, which couple to other effector proteins (like phosphodiesterases or ion channels), and metabotropic ligand binding is responsible for activating these associated G-protein pathways. Each activated metabotropic receptor can activate several hundred G proteins, and each activated G protein can then interact with several hundred effector proteins. This allows for a high degree of signaling amplification.

In summary, the greater flexibility and plasticity available to chemical synapses has made them the favored mode of synaptic transmission in the brains of vertebrates and invertebrates (e.g., the worm *C. elegans* has about 7000 chemical synapses but only 600 electrical synapses). For the rest of this chapter, the term *synapse* will be synonymous with the chemical synapse.

2. A MODEL SYNAPSE: THE NEUROMUSCULAR JUNCTION

The frog neuromuscular junction (NMJ) was the first synapse to be thoroughly investigated. It has many advantages for the study of synaptic transmission, including its ability for easy access, stimulation, and electrical recording. Figure 4 shows a schematic diagram of the frog NMJ. A single motor nerve axon terminates in several branches on a single muscle fiber. This area of multiple synapses is called the *end-plate region*. A recording electrode that impales the muscle fiber just underneath the end-plate region (electrode A in Fig. 4) will record a resting membrane potential of about -70 mV. In addition, several spontaneous miniature end-plate potentials (MEPPs or mini-EPSPs) will also be superimposed on the resting membrane potential. These electrical events were first recorded by Fatt and Katz in 1951. A recording electrode placed at some distance from the end-plate (electrode B in Fig. 4) will not detect these MEPPs because *electrotonic attenuation* reduces their already small amplitude (0.5 mV) to levels below the basal noise. Subsequently, in 1954 del Castilho and Katz noticed that the amplitudes of the MEPPs were remarkably consistent and that end-plate potentials (EPPs) frequently appeared as multiples of a standard size. The EPP therefore appeared to be composed of discrete units, or *quanta*, corresponding with unitary MEPPs. They called the standard MEPP amplitude the *quantal size* and denoted it with the symbol q .

Returning to Fig. 4, when a single AP was stimulated in the motor nerve, an end-plate potential was recorded by electrode A after a short delay. The shape of the EPP was similar to that of the MEPP, but its size was several-fold larger. The constant scaling factor between the EPP and MEPP was denoted by m , called the *quantal content* of the EPP. The EPP amplitude invariably exceeded the threshold for action-potential generation, so a muscle AP was also observed first at the end-plate (electrode A) and then further away (electrode B). Del Castilho and Katz further assumed that the end-plate contained several discrete sites for quantal release. Supposing that a number of these release sites, denoted N , were functional at any given time and had an average *probability of release* (P_r), they postulated that $m = NP_r$. This elegant statistical analysis of neurotransmitter release still shapes our modern quantitative views of synaptic function.

The morphologic correlates of q , m , and N are thought to be the synaptic vesicle, the number of vesicle fusions, and the number of functional active zones in the nerve terminal, respectively. Synaptic

vesicles are homogeneous in size (about 50 nm in diameter at the NMJ) and are believed to contain the same amount of neurotransmitter. Interestingly, certain mutants of the fruit fly *Drosophila* (called *lap* mutants) have unusually large synaptic vesicles and correspondingly larger MEPPs. At the frog NMJ, there are about 300 active zones. This redundancy, or high N value, allows for a small P_r at individual active zones but a large overall m value. In other words, there is a large safety factor at the NMJ so that it can function as a fail-safe relay to trigger muscle APs. One caveat to this general rule occurs when stimulation frequency is high. In this case, P_r is drastically reduced and failures in AP transmission can occur.

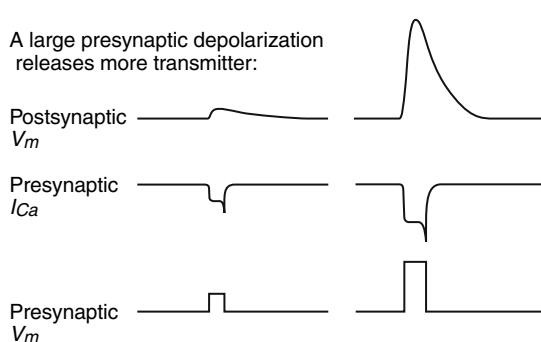
3. PRESYNAPTIC EXOCYTOSIS IS Ca^{2+} DEPENDENT

Neurotransmitter release occurs when synaptic vesicles fuse with the presynaptic plasma membrane. This process of exocytosis is triggered by the influx of free Ca^{2+} ions into the nerve terminal. Depolarization of the nerve terminal opens voltage-gated Ca^{2+} selective channels, and because calcium concentrations are much higher in the extracellular space than in the cytosol, calcium flows into the cell according to its electrochemical driving force. This flux of ions produces a current that can be measured, for example, using the *two-electrode voltage clamp* technique. This type of Ca^{2+} current recording was first demonstrated by Llinás and colleagues. When the postsynaptic neuron is impaled by a third electrode for recording EPSPs, it becomes possible to examine the relationship between a presynaptic Ca^{2+} current and a postsynaptic EPSP (Fig. 5). Small step depolarizations of the nerve terminal from -60 to -30 mV elicit a small, slow Ca^{2+} current and relatively small EPSP in the postsynaptic cell. A stronger presynaptic depolarization from -60 to 0 mV will evoke a larger, more rapid Ca^{2+} current and much larger EPSP. Similarly, short depolarizations will produce smaller EPSPs than will longer-duration depolarizations of the same magnitude. Using this method for comparing presynaptic and postsynaptic events, the relationship between calcium influx and postsynaptic response was found to be nonlinear.

Results from the squid giant axon synapse confirmed previous experiments in the frog NMJ showing that transmitter release depends exponentially on Ca^{2+} concentration in the extracellular medium (by a 4th power relationship). Physiologic Ca^{2+} ion concentrations are

Ca²⁺ Controls Transmitter Release

A. A large presynaptic depolarization releases more transmitter:



B. A long presynaptic depolarization releases more transmitter:

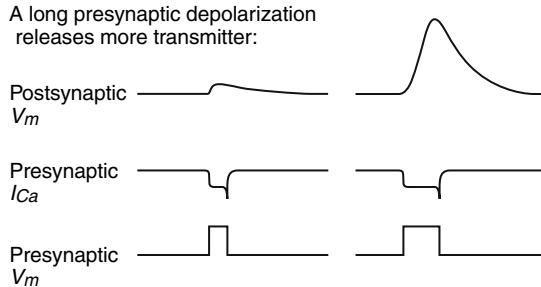


Fig. 5. Ca²⁺ ions and synaptic transmission. Schematic diagram of electrophysiologic recordings from the squid giant synapse. Simultaneous presynaptic and postsynaptic voltage-clamp recordings. (A) A step-like depolarization of the presynaptic terminal (bottom trace) causes the opening of voltage-gated Ca²⁺ channels and the activation of a presynaptic Ca²⁺ current. The resulting Ca²⁺ influx triggers transmitter release and a postsynaptic potential change in the postsynaptic cell. A small amplitude presynaptic depolarization elicits a small postsynaptic response, whereas a larger depolarization causes a larger Ca²⁺ current and a larger postsynaptic potential. (B) A short depolarization causes a short Ca²⁺ current and a brief and small postsynaptic potential, whereas a longer depolarization causes a longer Ca²⁺ current and a larger postsynaptic potential.

about 2 mM extracellularly, and intracellular free Ca²⁺ is around 100 nM. This imbalance results in a large electrochemical driving force toward calcium entry when Ca²⁺ channels are open, and local Ca²⁺ concentrations near the cytosolic mouth of these channels can reach levels as high as 100 to 300 μM for tens to hundreds of microseconds. Upon entry, calcium ions are thought to bind one or more proteins on docked synaptic vesicles, which act as a sensor for initiating the fusion process. The fusion sensor protein is thus activated by calcium for an extremely brief period, a fact that may help explain the extremely phasic or transient nature of neurotransmitter release.

One candidate protein for the Ca²⁺ fusion sensor is called *synaptotagmin*. It has two so-called C2 domains (similar to the protein kinase C [PKC] Ca²⁺-binding domain) that bind Ca²⁺. After binding calcium, synaptotagmin partially inserts itself into phospholipids of the plasma membrane to bind SNARE-type proteins crucial for vesicle fusion. SNARE proteins come in two varieties: vesicular (v-SNAREs) and target (t-SNAREs). The v-SNAREs are found on synaptic vesicles, whereas the t-SNAREs reside on the plasma membrane. In order for vesicle fusion to occur, v-SNAREs and t-SNAREs must associate to form a tight *core-complex* that is extremely resistant to unbinding. The SNARE core complex is highly energetically favorable and requires ATP hydrolysis for unbinding. It is believed that this complex serves as a mechanical hairpin that, when triggered, facilitates mixing of the synaptic vesicle and plasma membrane lipids for vesicle fusion. Evidence that SNARE proteins are essential for vesicle fusion comes from bacterial neurotoxins that selectively degrade SNARE proteins. These toxins are highly potent and require only a few molecules to completely block synaptic transmission. Botulinum toxin, now routinely used for cosmetic applications, is an example of such a compound.

Free Ca²⁺ may regulate other processes aside from vesicle fusion. For example, the recruitment of synaptic vesicles from reserve pools to the docked or *readily releasable pool* is accelerated by elevated intracellular Ca²⁺. Endocytosis, the process of synaptic vesicle reuptake, may also be regulated by Ca²⁺ at some synapses, and short-term facilitation of EPSPs can result from residual Ca²⁺ accumulation during a tetanus. In addition, Ca²⁺ activates different kinases and phosphatases, which regulate several forms of long-term morphologic and functional synaptic plasticity.

After synaptic vesicles fuse with the plasma membrane, they are recycled back into the nerve terminal (Fig. 3). The process of vesicular membrane reinternalization (or retrieval) from the plasma membrane is called *endocytosis*, and the synapse uses several forms of this process. Some endocytosis is very fast and occurs with a time constant of about 1 s. Other forms are slower (time constant of 10 to 20 s), and are probably mediated by clathrin-coated pits that form on the plasma membrane. Endocytosing vesicles require a GTPase called *dynamin* to “pinch off” from the plasma membrane into the intracellular space. Interestingly, the *Drosophila* mutant *shibire* has a temperature-sensitive defect in dynamin and becomes paralyzed at elevated temperatures (e.g., 29°C). Electron microscopy reveals that the nerve terminals of these paralyzed flies are

devoid of cytoplasmic synaptic vesicles. Furthermore, the plasma membrane is found to have a string of coated invaginations that cannot pinch off. This observation indicates that the terminals are incapable of completing endocytosis and cannot recycle their vesicular membrane after fusion. Accordingly, the surface area of the terminals is enlarged, and there are no vesicles available for continued exocytosis. This dramatic phenotype clearly demonstrates the importance of vesicle recycling for the continuous operation of a synapse. It also illustrates that severe *vesicle pool depletion* will block synaptic transmission.

4. NEUROTRANSMITTERS AND THEIR RECEPTORS IN THE MAMMALIAN BRAIN

A large proportion of synapses in the mammalian brain are excitatory and use the amino acid *glutamate* as their neurotransmitter. Glutamate is sequestered into vesicles by a *glutamate transporter* protein in the vesicular membrane. Synaptic vesicles are acidic ($\text{pH} = 5.7$), and the energy from their proton concentration gradient is used to transport neurotransmitter into the vesicle. Synaptic vesicles therefore require a proton ATPase to acidify their interior (or lumen). There are two broad categories of synaptic vesicle proteins: transport proteins (e.g., proton pumps, $\text{Na}^+/\text{Ca}^{2+}$ exchangers, and Cl^- ion transporters) and trafficking/fusion proteins (e.g., v-SNAREs, synaptotagmin, synapsin). Once glutamate is released into the synaptic cleft, it diffuses away quickly (within milliseconds) and binds to *plasma membrane glutamate transporters* located in presynaptic and postsynaptic neurons and glia (or astrocytes). These transporters use existing sodium and potassium gradients to drive glutamate back into the cytoplasm. Neurotransmitter is recycled in this manner, and because excessive glutamate is toxic for neurons and can lead to cell death, the external glutamate concentration is tightly controlled by this reuptake process.

On the postsynaptic cell, glutamate receptors can be classified into two general types: ionotropic and metabotropic receptors. As noted previously, ionotropic receptors directly gate ion channels, whereas metabotropic receptors are coupled to G proteins. There are three kinds of ionotropic glutamate receptors, each named after the glutamate analogue they bind preferentially: α -amino-3-hydroxy-5-methyl-4-isoxalane propionate (AMPA), N-methyl-D-aspartate (NMDA), and kainate. Glutamate and the synthetic compound AMPA are potent *agonists* for the AMPA-type receptor. The AMPA receptor also has specific *antagonists* such as the compounds 6-cyano-

7-nitroguinoxaline-2,3-dione (CNQX) and 6-nitro-7-sulphamobenzoquinoxaline-2,3-dione (NBQX). These do not affect the NMDA receptor. Glutamate binding to the AMPA receptor opens a nonselective cation channel permeable to both Na^+ and K^+ ions; an event that tends to bring a negative resting membrane potential toward 0 mV. AMPA receptors have intrinsically fast kinetics and desensitize within milliseconds given a continuous pulse of glutamate. The fast EPSPs observed at excitatory synapses are mediated by AMPA receptor activation. NMDA receptors have slower kinetics, use glycine as a co-agonist, and do not desensitize quickly. They are often colocalized at the PSD with AMPA receptors. NMDA and kainate receptors have also been found recently in some CNS presynaptic nerve terminals, but their function is not well understood.

The major inhibitory neurotransmitters in the brain are *GABA* and *glycine*. These transmitters are similarly packaged into vesicles by vesicular GABA/glycine transporters expressed on the membrane of synaptic vesicles. Other neurotransmitters in the mammalian brain include acetylcholine, ATP, adenosine, and several amine transmitters (e.g., dopamine, noradrenaline [or norepinephrine], adrenaline [or epinephrine], serotonin, and histamine). The *catecholamine* transmitters (dopamine, noradrenaline, and adrenaline) are all synthesized from the essential amino acid tyrosine in a common biosynthetic pathway. Catecholamines are important in the brain, not only as neurotransmitters, but also as *neuromodulators* that have widespread effects on neuronal circuits. Interestingly, vesicular amine transporters are targets for several pharmacologic agents. For example, the antipsychotic drugs reserpine and tetrabenazine inhibit amine transporters, and the psychostimulants amphetamine and “ecstasy (3-Y-methylenedioxy-N-methylamphetamine)” are thought to dissipate the pH gradient of synaptic vesicles containing amine transmitters.

There are three types of GABA receptors, termed GABA_A , GABA_B , and GABA_C . The GABA_A and GABA_C receptors are ionotropic, whereas the GABA_B receptor is metabotropic. The GABA_A receptor is blocked by bicuculline and desensitizes quickly, whereas GABA_C receptors desensitize much more slowly and are insensitive to bicuculline. GABA binding to the GABA_A or GABA_C receptor opens an anion-selective Cl^- channel that tends to bring the membrane potential toward the equilibrium potential of Cl^- (about -60 to -80 mV, depending on intracellular Cl^- concentration). This hyperpolarization usually inhibits the postsynaptic neuron from firing APs. GABA_B receptors are also known to have

inhibitory function. For example, the GABA_B receptors of some presynaptic terminals inhibit Ca²⁺ channels and cause a reduction of transmitter release. Glycine receptors are ionotropic (anion-selective Cl⁻ channels) and can have very rapid kinetics of activation and deactivation.

5. THE INTERPLAY OF EXCITATION AND INHIBITION

A simple neural circuit that demonstrates how excitatory and inhibitory synapses are combined to produce a functionally significant behavior is the myotatic (or “knee-jerk”) spinal reflex (Fig. 6). When a hammer is tapped on the extensor muscle, sensory axons carry the information from the extensor muscle toward the spinal cord. These or any other axons that carry information to the brain or spinal cord are called *afferents*. Motor axons, or *efferents*, carry information away from the brain or spinal cord and initiate a behavioral response to the hammer tap (an upward jerk of the leg). Along their path, the sensory afferents branch to make synaptic contact with both motor neurons of the ventral horn and spinal *interneurons* (i.e., neurons that lie entirely in the spinal cord). The sensory axon synaptic terminals release glutamate and are excitatory, whereas the interneurons release

inhibitory neurotransmitters (GABA and glycine). The sensory neurons thus excite motor neurons that make synaptic contact with the extensor muscle and cause it to contract. At the same time, the sensory axon terminals also excite local inhibitory interneurons that synapse onto the motor neurons innervating the flexor muscle. This causes a relaxation of the flexor and permits the opposing extensor muscle to dominate the behavioral response.

Electrophysiologic recordings from the sensory, interneuron, and motor neurons of the myotatic spinal circuit provide insight into how the circuit operates (Fig. 6). There are several types of electrophysiologic recordings. *Extracellular recording* with an electrode (usually metal) placed near the neuron measures the all-or-nothing APs produced by the neuron. *Intracellular recordings* where the electrode (usually a glass micropipette filled with a conducting solution) impales the cell (like in Fig. 4) can detect smaller, subthreshold synaptic potentials. *Patch-clamp recordings*, where a glass electrode is placed on the membrane of the cell, are very low noise recordings that can even detect single channel currents. Examples of extracellular recordings from spinal neurons involved in the myotatic reflex are shown in Fig. 6. Neurons usually have a low level of spontaneous AP firing, even in the absence of any synaptic or sensory input. The hammer tap to

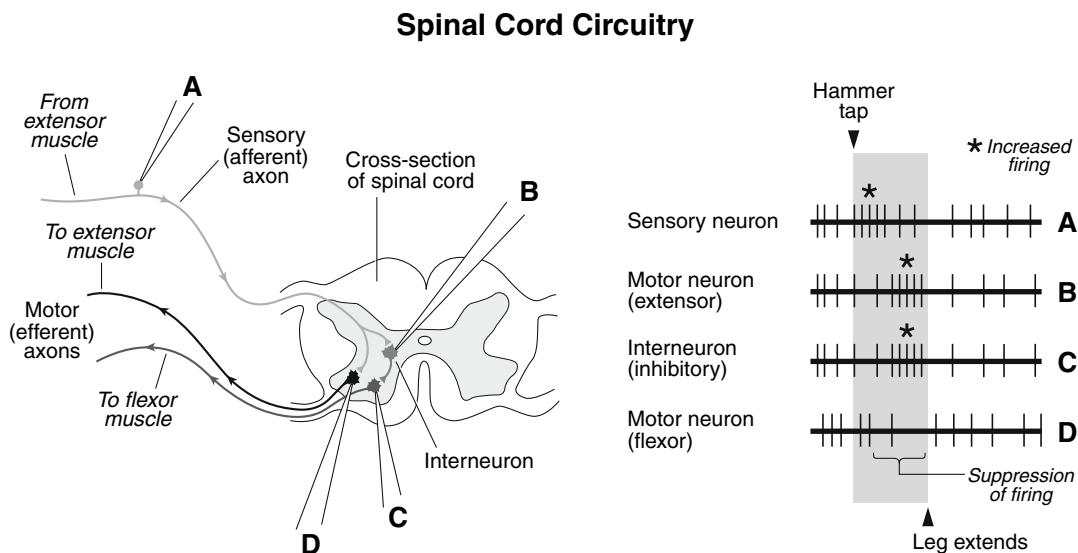


Fig. 6. A reflex circuit in the spinal cord. Schematic diagram of spinal cord circuitry. Extracellular electrophysiologic recordings from the four specified neurons are shown on the right-hand panel. A gentle hammer tap to the knee causes a burst of APs to travel along the sensory axons (recorded by electrode A) and toward the neurons of the spinal cord. The sensory axon branches into two pathways exciting the motor neurons connected to the extensor muscle (recorded by electrode D) and certain interneurons in the spinal cord (recorded by electrode B). The interneurons then inhibit the firing of the motor neurons connected to the flexor muscle (recorded by electrode C). The hammer tap thus causes the reflex of leg extension. (Modified from Augustine, G.A., Chapter 1. In: *Neuroscience*, edited by Purves et al, 2001.)

the knee elicits a burst of APs in the sensory neuron, which is followed after a brief delay by a burst of APs in the motor (extensor) neurons and interneurons. The interneurons then inhibit the firing of the flexor motor neurons. The end result is a leg extension. Recordings of AP onset, duration, and frequency thus provide a real-time picture of neuronal activity. By using the complementary intracellular and patch-clamp recording techniques, the mechanisms underlying circuit function can be determined.

6. SYNAPSES ARE HETEROGENEOUS AND CAN BE SPECIALIZED

Synapses can exhibit different functional properties and architectures depending on their specific information transfer and processing needs. We will next discuss ribbon-type and calyx-type synapses found in the retina and brain stem as examples of highly specialized synapses. We then summarize some of the hallmark properties of the more typical bouton-type synapses found in the cortex and other parts of the brain.

Ribbon-type synapses are found in the vertebrate retina and the cochlea. Retinal photoreceptors and bipolar cells and cochlear hair cells transmit sensory information via this type of synapse. Light and sound stimuli produce tonic and graded membrane depolarizations in these cells, which elicits the graded release of neurotransmitter from the specialized active zones called *synaptic ribbons*. These synapses are specialized to transmit the large amounts of sensory information involved in vision and hearing.

One of the techniques used to study the synaptic release of neurotransmitter from these cells is time-resolved *membrane capacitance measurements*, which are based on the patch-clamp recording technique. Electrical membrane capacitance is proportional to the surface area of a cell. When there is an increase in cell surface area (e.g., during exocytosis), membrane capacitance increases. Similarly, when surface area decreases (e.g., during endocytosis), membrane capacitance decreases. Net changes in cell surface area of <1% can be detected by this exquisitely sensitive technique. An example of a membrane capacitance (C_m) measurement is shown in Fig. 7. This C_m measurement was obtained from the whole-cell patch-clamp recording of an isolated synaptic terminal of a goldfish retinal bipolar cell. The terminal's baseline C_m value is constant until a step depolarization (from a holding potential of -60 mV to -10 mV) is given during the gray bar (Fig. 7A). This depolarization opens

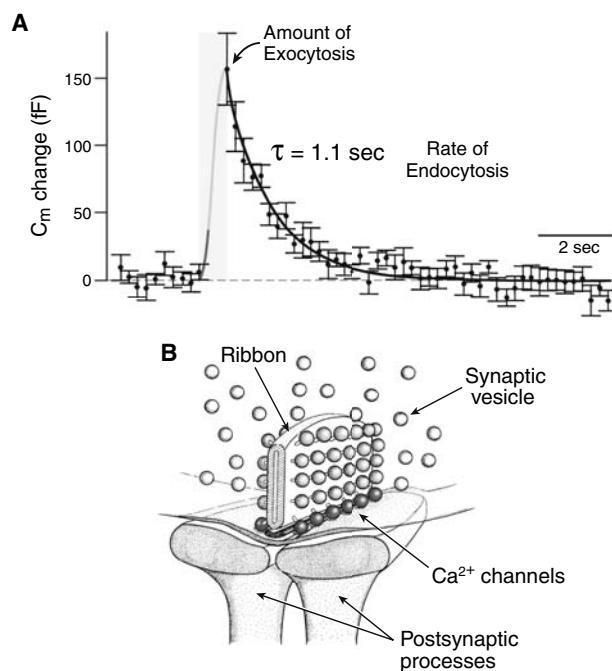


Fig. 7. Synaptic transmission at a ribbon synapse. (A) Membrane capacitance (C_m) measurements from the ribbon-type synaptic terminal of bipolar cells in the goldfish retina. A step depolarization (from the holding potential of -60 mV to -10 mV), given during the period marked by a gray bar, elicits a jump in C_m of about 150 femtofarad (fF). This corresponds with the fusion (or exocytosis) of about 6000 synaptic vesicles with the presynaptic plasma membrane. After the jump in C_m , the capacitance decays back to the original baseline following a single exponential time course. The time constant $\tau = 1.1$ s is a measure of the rate of membrane retrieval (endocytosis). (B) A three-dimensional schematic drawing of the ribbon-type active zone of bipolar cell synaptic terminals. The electron-dense “ribbon” sheet has a halo of tethered synaptic vesicles. Vesicles located in the bottom row (black vesicles) are docked to the plasma membrane and are close to Ca^{2+} channels. They constitute a readily releasable pool of vesicles. The other vesicles (gray vesicles) tethered to the ribbon structure are thought to be next in line for fusion. (Modified from von Gersdorff, H. Synaptic ribbons: versatile signal transducers. *Neuron* 2001;29:7–10).

Ca^{2+} channels, and the resulting influx of Ca^{2+} into the terminal triggers the fusion of synaptic vesicles with the plasma membrane along with an increase in the terminal's surface area. This is detected as a jump in C_m immediately after the depolarization. The size of the jump is 150 femtofarad. Because each synaptic vesicle has a diameter of about 30 nm and a capacitance of 26.4 attofarad, the C_m jump corresponds with the fusion of about 6000 synaptic vesicles. A strong depolarization can thus elicit the fusion of several thousand vesicles at ribbon synapses. Large vesicle

pools that can be released quickly are a characteristic of ribbon-type synapses, and this property allows them to release small or large amounts of transmitter depending on the degree and speed of the presynaptic depolarization. After the jump in C_m , membrane capacitance decays back to the original baseline following a single exponential time course. The exponential time constant of $\tau = 1.1$ s is a measure of the rate of membrane retrieval (endocytosis). Fused synaptic vesicle membrane can thus be quickly reinternalized and recycled into the terminal.

Figure 7B diagrams the unique architecture of a ribbon-type active zone as seen by electron microscopy. An electron-dense “ribbon” sheet has a halo of tethered synaptic vesicles. Vesicles located in the bottom row (black vesicles) are docked to the plasma membrane. They are close to Ca^{2+} channels and presumably poised for rapid exocytosis, constituting an immediately releasable pool of vesicles. The other vesicles (gray vesicles) tethered to the ribbon structure are thought to be next in line for fusion. After these vesicles fuse, the synapse is refractory to subsequent release for a few seconds. The vesicles tethered to the synaptic ribbon may thus be the morphologic correlate of a *readily releasable pool* of synaptic vesicles.

A second type of specialized synapse is located in the mammalian auditory brain stem, where *calyx-type synapses* involved in calculating the location of sound sources are found. The morphology of the synapse is shown in Fig. 8. The large calyx-type synaptic terminal has hundreds of small *conventional active zones* in adult animals. Each conventional active zone has about 2 to 10 docked vesicles and a cluster of reserve vesicles, as depicted in Fig. 2 and Fig. 3. At the calyx of Held, a single presynaptic AP evokes the rapid release of about 200 to 300 synaptic vesicles. This produces a large EPSC and EPSP that safely cross the threshold for the postsynaptic AP (Fig. 9). Thus synaptic transmission is very safe (free of spike failures) and fast at this specialized synapse. In addition, each conventional active zone in the calyx of Held terminal has a different release probability. During a stimulus consisting of a train of presynaptic APs, this heterogeneity of release probabilities means that some active zones (with high release probability) will release transmitter early during the stimulus train, whereas others (with low release probability) will release late in the stimulus train. This synapse is thus able to faithfully follow a presynaptic train of APs, even when this train occurs at very high frequency (e.g., 800 Hz). The large number of active zones also ensures a short synaptic delay

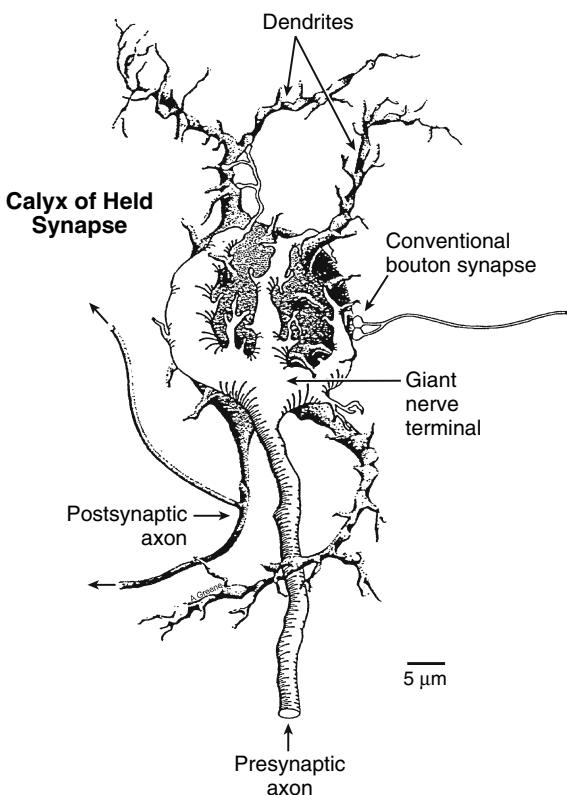


Fig. 8. The calyx of Held synapse. A diagram of the adult calyx of Held, a glutamatergic nerve terminal in the mammalian auditory brain stem. Notice the large-caliber axon (4 to 12 μm) that gives rise to the calyx terminal. The postsynaptic cell has relatively short dendrites and an axon with a collateral branch. A typical bouton-type terminal is indicated for comparison. Most synapses in the brain are conventional bouton-type synapses formed on postsynaptic dendrites. (Modified from Morest, D.K. et al. Stimulus coding at the caudal levels of the cat's auditory nervous system. In: *Basic Mechanisms of Hearing*, edited by Möller, A.R. Academic Press, New York, 1973.)

because the EPSP is very fast and large (Fig. 9 and Fig. 10). This leads to a precise preservation of AP timing. The ability of the postsynaptic neuron to follow presynaptic APs with high fidelity and short synaptic delays are features that help in the processing of sound localization in this auditory pathway synapse.

Some synapses, like the neuromuscular junction, the calyx-type brain-stem synapse, and the squid giant synapse, are relatively large and guarantee fail-safe transmission. In adult animals, these synapses are relatively fixed in their transmission characteristics. Most synapses in the mammalian brain are, however, more plastic and physically small (diameter of $<1 \mu\text{m}$; Fig. 8). They produce small EPSPs and have a low release probability. This makes them rather unreliable

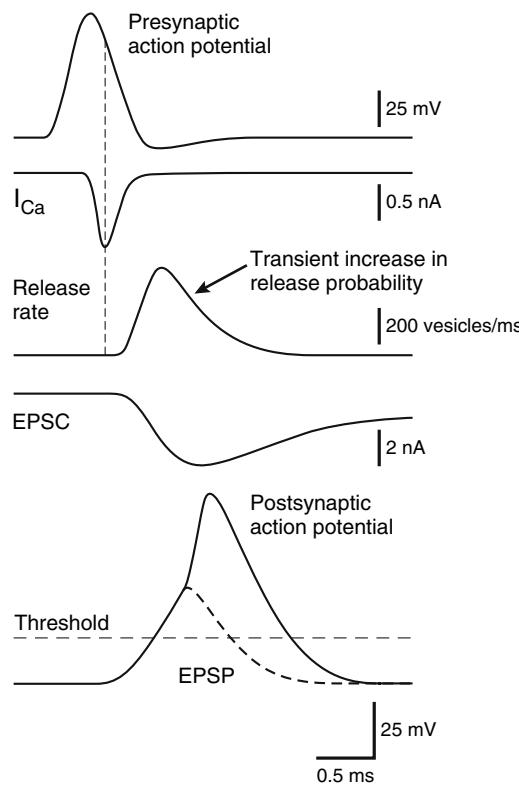
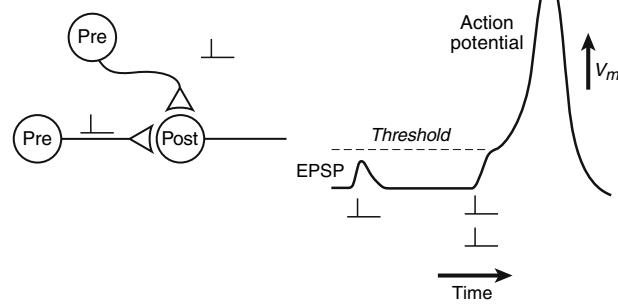


Fig. 9. Synaptic transmission at a conventional active zone synapse: the calyx of Held. A presynaptic AP in the calyx nerve terminal triggers the opening of voltage-dependent Ca^{2+} channels, which cause a Ca^{2+} current that occurs during the down-stroke phase of the AP. Ca^{2+} influx triggers the fusion of synaptic vesicles that release glutamate into the synaptic cleft. The rate of glutamate release (or vesicle fusion) rises rapidly after the Ca^{2+} current and then decays rapidly. The time between the peak of the Ca^{2+} current and the peak of the release rate is 0.5 ms. Glutamate binding to postsynaptic receptors opens ion channels in the postsynaptic membrane that are permeable of Na^+ and K^+ ions (the postsynaptic receptors are called AMPA-type glutamate receptors). This ion influx generates an excitatory postsynaptic current (EPSC) that depolarizes the postsynaptic neuron creating a fast and large excitatory postsynaptic potential (EPSP). The time between the peak of the release rate and the peak of the EPSC is 0.4 ms. The large EPSP quickly crosses the AP threshold. Thus a postsynaptic AP is generated in the postsynaptic neuron without failure and with minimal synaptic delay (Modified from von Gersdorff, H. and Borst, J.G.G. Short-term plasticity at the calyx of Held. *Nat Rev Neurosci* 2002; 3: 53–64.)

in transmitting signals. These synapses are called conventional *bouton-type synapses*. Their small EPSPs may, nevertheless, summate on a postsynaptic neuron to help it fire APs (Fig. 10). The relative timing of presynaptic APs and resulting EPSPs is critical for *spatial* or *temporal summation* (Fig. 10). The strength with which a postsynaptic neuron will be stimulated will thus depend on the individual strength of the

Spatial and Temporal Summation

A. Spatial summation



B. Temporal summation

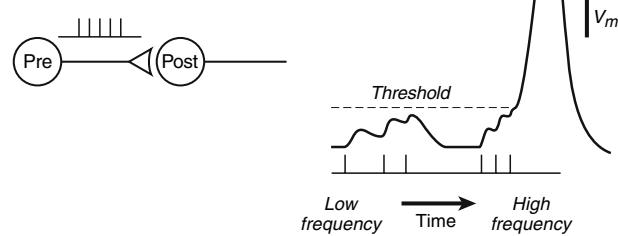


Fig. 10. Spatial and temporal summation at bouton-type synapses. **(A)** Spatial summation. Schematic diagram showing how the input from two small, bouton-type presynaptic terminals can summate to produce a suprathreshold excitatory postsynaptic potential (EPSP). The input from just one bouton is not enough to trigger an AP in the postsynaptic cell. However, if the two inputs elicit simultaneous EPSPs in the postsynaptic cell, the summed depolarization is enough to trigger an AP. **(B)** Temporal summation. Low-frequency stimulation may not be enough to trigger an AP in the postsynaptic cell. However, high-frequency stimulation may cause the individual EPSPs to summate fast enough to trigger an AP. The importance of the relative timing of EPSPs is thus evident from these two examples. (Modified from Gardner, D. Synaptic transmission. In: *Neuroscience in Medicine*, edited by Conn, M.P. J.B. Lippincott, Philadelphia, 1995.)

separate bouton-type synapses that make contact with its dendrites and soma and the relative timing of transmitter release. Whether an EPSP generates a spike (AP) or not depends on the amplitude of the EPSP and the *spike threshold* of the postsynaptic neuron. In addition, the nature of the transmission (excitatory or inhibitory) depends on the neurotransmitter type (e.g., glutamate [Glu] is excitatory and GABA is inhibitory), the postsynaptic ionotropic receptor type (e.g., glutamate or GABA receptor ion channels), and on the reversal potentials for the ionic currents present on the postsynaptic neuron (Fig. 11). The reversal potentials for the EPSC (mediated by Glu receptors, which are permeable mainly to Na^+ and K^+ ions)

Action potential threshold and reversal potentials

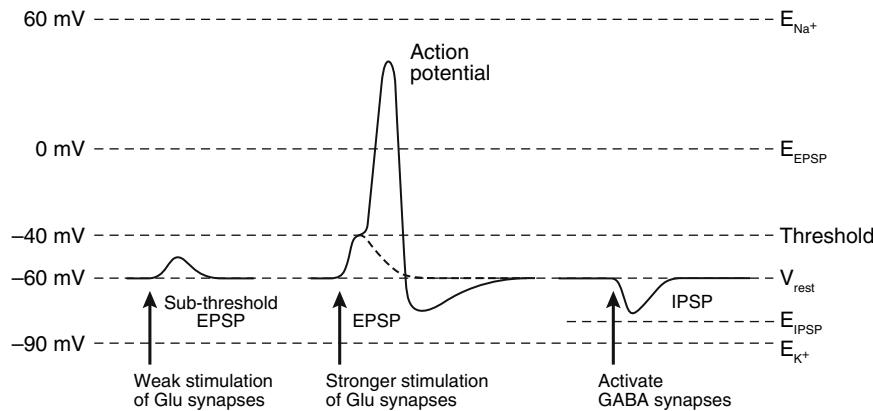


Fig. 11. Synaptic transmission and reversal potentials: excitation and inhibition. A subthreshold excitatory postsynaptic potential (EPSP) is elicited in a postsynaptic neuron. This can be generated by a single excitatory volley or by the recruitment of only a few axons and/or synapses onto the postsynaptic neuron. The recruitment of more afferent axons with stronger stimulation causes more excitatory glutamatergic synapses to release glutamate (Glu). Likewise, the more coincident activation of several Glu synapses can generate a larger and faster EPSP, which reaches the threshold for AP firing. The reversal potential for the EPSP (E_{EPSP}) is located near 0 mV because the AMPA-type and NMDA-type receptors that mediate the EPSP are transmitter-gated ion channels that are equally permeable to Na^+ and K^+ ions. Activation of GABAergic synapses generates an inhibitory postsynaptic potential (IPSP) that has a reversal potential (E_{IPSP}) at around -80 mV because GABA receptors are transmitter-gated ion channels selectively permeable to Cl^- ions. In this example, the neuron's resting membrane potential (V_{rest}) is -60 mV, and the threshold for AP firing is -40 mV, which is where Na^+ channels begin to open significantly with higher probability. The Nernst reversal potential (or equilibrium potential) for Na^+ ions (E_{Na}) is $+60$ mV and for K^+ ions (E_{K}) is -90 mV. Because $E_{EPSP} > \text{threshold}$, glutamate is an excitatory neurotransmitter, whereas $E_{IPSP} < \text{threshold}$ makes GABA an inhibitory neurotransmitter. Note how the IPSP tends to inhibit the triggering of APs. GABAergic synapses thus tend to reduce the excitability of adult mammalian neurons.

and the IPSC (mediated by GABA receptors, which are selectively permeable to Cl^- ions) will in their turn depend on the concentration of Na^+ , K^+ , and Cl^- ions in the inside and outside of the synapse (Fig. 11; see also Chapter 3).

Bouton-type synapses of the CNS can also change their properties dramatically after certain patterns of neuronal activity. This aspect of synaptic plasticity is a way of storing new information acquired by experience in the synaptic strength of a particular synapse. The small size of bouton-type synapses also allows for a large number of synapses to be packed in a small volume, thus increasing the computational capacity of the brain. Often, one presynaptic bouton-type terminal synapses onto one spine-like dendritic structure of a postsynaptic neuron (as depicted in Fig. 3), and a single dendrite can have hundreds to thousands of *postsynaptic spines* (see Fig. 2 for an electron microscope image of a spine). Each bouton-type terminal typically has one conventional active zone with about 2 to 10 docked vesicles and a cluster of reserve vesicles in its cytosol. In total, the whole bouton has about 200 vesicles. Accordingly, these synapses have a small readily releasable pool estimated to be about

10 vesicles. Neuromodulators, such as noradrenaline and dopamine, can also change the output of bouton-type synapses, making them especially flexible transducers of information.

7. SHORT-TERM AND LONG-TERM SYNAPTIC PLASTICITY

After a train of presynaptic APs (or a tetanic stimulus), the corresponding postsynaptic potentials can grow in amplitude (a process called *short-term facilitation*) or decrease in amplitude (a process called *short-term depression*). Often, synapses display first short-term facilitation followed by short-term depression or, alternatively, just short-term depression. There are multiple synaptic mechanisms that may underlie these forms of short-term synaptic plasticity. For example, after the first presynaptic AP in a stimulus train, the Ca^{2+} concentration in the terminal decays back to resting levels. However, this decay may take several milliseconds to be completed depending on the Ca^{2+} buffering capacity of the terminal. The second stimuli in the train may thus elevate Ca^{2+}

concentrations to levels higher than that of the first stimulus. Given the highly nonlinear dependence of transmitter release on Ca^{2+} , short-term facilitation may therefore be due to the high resting Ca^{2+} concentrations reached during a stimulus train.

Short-term depression may be caused by presynaptic or postsynaptic mechanisms. During a stimulus train, the pool of readily releasable vesicles may get depleted at a rate faster than the replenishment rate by newly recruited vesicles. The resulting depletion of the readily releasable pool of vesicles will thus cause synaptic depression. The presynaptic Ca^{2+} current may also become progressively inactivated during a stimulus train leading to decreased release. Alternatively, postsynaptic factors may also lead to reduced EPSPs or IPSPs, as postsynaptic ionotropic receptors may desensitize and/or saturate (i.e., the receptors may become insensitive to further neurotransmitter release). Stimulation at very high frequencies often leads to short-term depression. Thus, high-frequency inputs may be strongly filtered from a neuronal circuit by short-term plasticity.

Some synapses can change their release characteristics for a period of hours to days after receiving a particular stimulus pattern. This form of long-term plasticity is commonly found in the mammalian hippocampus and cortex. Synaptic strength may thus be increased (a process called *long-term potentiation*) or reduced (a process called *long-term depression*). One mechanism for long-term potentiation involves the properties of the AMPA- and NMDA-type receptors located on postsynaptic dendritic spines. These receptors bind glutamate and then open channels permeable to cations. The release of glutamate from bouton-type presynaptic terminals thus produces a rapid AMPA receptor-mediated EPSPs that transiently depolarizes the spine. Glutamate binds also to the slower activating and inactivating NMDA receptors colocalized with the AMPA receptors on the spine. However, when the spine is initially at its resting membrane potential, external Mg^{2+} ions block the NMDA channel and no current flows through the channel, even as its transmitter-activated gate is slowly opened by glutamate. On the other hand, if the dendritic spine has been previously depolarized

by other nearby synaptic inputs, the Mg^{2+} block is removed and the NMDA channel pore can now pass current upon glutamate binding. The NMDA receptor is thus both a voltage-dependent and ligand-gated channel, with the voltage dependence arising from the voltage-dependent Mg^{2+} block of the channel pore. NMDA receptors, unlike most AMPA receptors, are permeable to Ca^{2+} ions. Thus the NMDA receptor-mediated postsynaptic current will increase Ca^{2+} concentrations in the spine, leading to the activation of Ca^{2+} -dependent kinases that may phosphorylate the AMPA receptor and thus augment its current. In addition, Ca^{2+} ions in the spine may trigger the release of retrograde messengers that feed back onto the bouton terminal to increase its release probability. These molecular events may thus produce long-term changes in synaptic strength. The NMDA receptor can thus operate as a *coincidence detector* that detects spine depolarization by other synaptic inputs and glutamate release from its own opposing bouton terminal. This association of different synaptic inputs, which are activated at specific times, and the resulting selective strengthening of a particular synapse may constitute a cellular mechanism for learning and memory.

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Robert D. Grubbs

CONTENTS

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AND STRUCTURAL/MECHANISTIC
- RECEPTOR STRUCTURE AND FUNCTION
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- CONCLUSION
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1. RECEPTOR CLASSIFICATION SCHEMES: ANATOMIC, PHARMACOLOGIC, AND STRUCTURAL/MECHANISTIC

Classification of cell signaling receptors has evolved in stages as the technology available for differentiating them has improved. The earliest classification systems were anatomic, based on the location of specific types of receptors in various tissues. Examples include somatic or autonomic, parasympathetic or sympathetic, and postsynaptic or presynaptic (e.g., having the receptor on the nerve terminal or dendrite and serving neuromodulatory function) classification systems.

The pharmacologic classification of receptors has been developing since the late 1800s, when the first transmitter substances were isolated and chemically characterized. Using this scheme, receptors are classified according to transmitter groups and their response to drugs. Receptors that respond to the catecholamines (e.g., dopamine, norepinephrine, and epinephrine) are known as catecholaminergic or sometimes “adrenergic” receptors. Because some respond better to norepinephrine than to epinephrine, they have been further subdivided into α - and β -adrenergic receptors. Both of these receptor subtypes have been further characterized into types 1 and 2 (e.g., β_1 , β_2). These subtypes can be demonstrated pharmacologically by differences in the

rank order of potency of a set of compounds with relatively small chemical differences in their structures. For example, the rank order of potency to compounds X, Y, and Z at adrenergic subtype β_1 receptors might be $X > Z > Y$, whereas for adrenergic subtype β_2 receptors, it would be $Z > Y > X$. Although these subtle differences have made it necessary for the student to learn a multitude of receptor subtypes, the importance of knowing the nature and distribution of receptor subtypes for the purposes of understanding drug therapeutic and adverse effects cannot be overemphasized. For example, it is possible to develop a drug (e.g., β_2 -adrenergic agonist) that dilates bronchial smooth muscle in an asthmatic that does not have significant effects on the β_1 receptor of the heart, which would greatly increase the heart rate and cause cardiac palpitations.

The structural/mechanistic method for the classification of receptors is based on information obtained from the cloning and sequencing of genes for receptors. Cloning of genes refers to the process of inserting copies of the gene for a specific protein (e.g., a receptor) into bacteria and allowing the natural reproduction and growth of the bacteria to produce the gene, mRNA, or protein of interest for harvest in bulk form.

Using this classification scheme of receptors, four groups or “families” of receptors have been revealed, each with a different molecular mechanism that activates the cell to respond after the transmitter has combined with its receptor. One of the major families includes the ion channel-gated receptors. These receptors form a channel or pore in the membrane

through which various ions can travel. Transmitter coupling can open or close the membrane pores.

Another structural/mechanistic family of receptors is the G protein-coupled receptors (GPCRs). The receptor protein, which spans the membrane seven times, activates a G protein, so named because it binds to and eventually hydrolyzes guanosine 5' triphosphate (GTP). This protein initiates a series of events (e.g., second-messenger system) that produces an effect within the cell, such as the opening or closing of an ion channel, the production of another second messenger, such as cyclic AMP, or the stimulation of genetic translation and transcription.

Although it seems logical that a given member of a pharmacologic class of receptors (e.g., cholinergic) would fall within the same gene family of the structural/mechanistic class of receptors, this is not always the case. The nicotinic cholinergic receptor belongs to the ion-gated family of receptors, and the muscarinic cholinergic receptor, which also responds to acetylcholine, belongs to the *GPCR* family. Thus, the muscarinic cholinergic receptor and the adrenergic receptor that responds to norepinephrine belong to the same superfamily of membrane proteins.

Other receptor families include the membrane receptors that respond to growth factors and the intracellular cytoplasmic receptors that respond to the steroid hormones (e.g., estrogen, testosterone, and cortisone) and thyroxine. The membrane-bound receptors that respond to growth factors have intrinsic enzymatic activity that is responsible for initiating their cellular response. The cytoplasmic portion of these receptors contains a tyrosine kinase domain that autophosphorylates the dimerized receptor, permitting it to activate a signaling cascade through the activation of one of the small G proteins, such as Ras. Although receptors for several growth factors have been identified in the brain, their precise role has not yet been determined.

In this chapter, specific receptors are discussed within the framework of the pharmacologic method of classification. This method is important in terms of medical practice and is the basis for therapeutics. However, receptors will be further defined in terms of their structural/molecular mechanisms and the major gene families to which they belong.

2. RECEPTOR STRUCTURE AND FUNCTION

A receptor is a cell component, usually a protein that binds to a drug, a transmitter, or a hormone to alter the cellular response. Receptors are like enzymes that bind to a substrate and catalyze a cellular activity. In the case of enzymes, the activity is usually the

addition or deletion of an atom or group of atoms to or from the substrate. In the case of the receptor, the transmitter is not chemically changed. These membrane-bound proteins, when activated by their transmitters, catalyze an activity within the membrane, which may be the opening of an ion channel or regulation of an intracellular signaling pathway that alters the biological state of the cell.

The concept of the cellular receptor was developed in the early 1900 s by J.N. Langley, who ascribed the effects of drugs and hormones to an interaction with a receptive substance (now known as receptor). Later, A.J. Clark and other scientists, including A.V. Hill and J.H. Gaddum, mathematically described these interactions and developed methods for characterizing them pharmacologically.

The attractive forces between the transmitter or drug and its receptor can be chemically mediated by covalent, ionic, hydrogen binding, or van der Waals forces. High-energy covalent bonding is rare, occurring only with a few drugs such as the α -adrenergic receptor blocker phenoxybenzamine. This type of binding is irreversible, noncompetitive, and is not used by any transmitter. Ionic attractions occur between cationic (e.g., positive) and anionic (e.g., negative) charges of different molecules and provide an attractive force that enables the transmitter to find the receptor. Hydrogen bonding is the attraction between hydrogen atoms and unpaired electrons in nitrogen or oxygen atoms and is important for attracting the ligand to the receptor. Van der Waals forces are weak dipolar interactions, but because of the number of atomic interactions and the complementarity or “fit” between the ligand and receptor, these forces are important for defining the tightness of binding (e.g., affinity) and specificity of the receptor for its transmitter. They are also important in determining the effect of binding by defining the conformational change that the receptor undergoes and thus the nature of the biological effect produced (agonistic and antagonistic).

2.1. Receptors Can Be Mathematically Characterized Using Biochemical Kinetic Parameters Developed to Understand Enzymes

The interactions of most transmitters with their receptors follow Michaelis-Menten kinetics, as do biochemical enzymatic reactions. Figure 1 shows two curves generated by measuring the binding of a transmitter such as acetylcholine to its receptor. This could be the effect of increasing concentrations of acetylcholine on the firing rate of a neuronal pathway in the forebrain. In the graph on the left (often called a

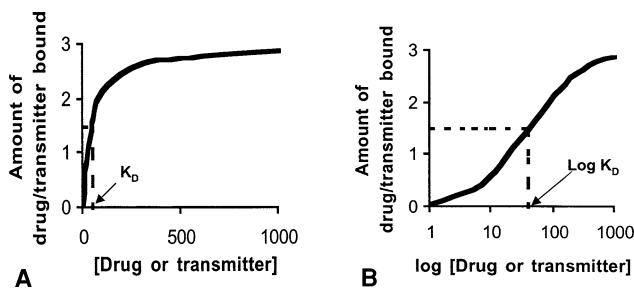


Fig. 1. (A) Graph of a Michaelis-Menten type plot in which the amount of a labeled drug or transmitter bound to receptor is plotted against the concentration of drug or transmitter in the assay. The concentration that produces 50% of the maximal receptor occupancy (B_{\max}) represents the dissociation constant (K_d). (B) Graph of a typical concentration-dependent binding curve in which the amount bound is plotted against the log of the concentration. In this case, the concentration that produces half of the B_{\max} is the log of the K_d .

Michaelis-Menten curve), increasing concentrations of the transmitter lead to an increased response that eventually plateaus. This plateau has been shown by kinetics to represent the maximal number of receptors present in the tissue sample, B_{\max} . The transmitter concentration that produces one-half of the maximal binding possible in this preparation yields a number known as the K_d , or dissociation constant. This number describes the affinity of the receptor and is defined as the amount of substrate needed to activate or occupy one-half of all the receptors. If this number is low (e.g., in the pM or nM range), the receptor-transmitter interaction is said to be a high-affinity interaction. If the concentration of the transmitter needed to cause one-half of the maximal effect is high (e.g., in the μM or mM range), the receptor is said to have a low affinity for this particular substance.

The graph in Fig. 1B represents the same data shown in Fig. 1A, only with the x -axis modified so that the logarithm of the transmitter concentration is plotted against the amount of transmitter bound to the receptor. This method, commonly called the log dose-response plot, consistently yields a sigmoidal curve and is useful for characterizing pharmacologic interactions, providing the log of the K_d . Although the Michaelis-Menten or direct plot and the log dose-response plot provide a good first approximation of the affinity of transmitter-receptor interaction, it is difficult to obtain precise estimates of the K_d or the B_{\max} directly from these plots because they are nonlinear. To obtain an accurate estimate of these binding parameters, the Scatchard plot and the Hill plot are often used. Figure 2 shows representative examples of these plots.

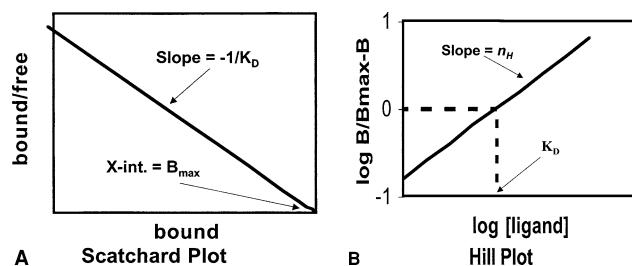


Fig. 2. (A) The graph represents a typical Scatchard plot. The ratio of the amount of a radiolabeled ligand or transmitter bound to a membrane preparation over the total free amount of radiolabeled ligand added is plotted against the amount of ligand bound. This plot provides the total number of receptors (B_{\max}) and their dissociation constant (K_d), which is determined from the slope. If the response is curvilinear (dotted line), it indicates that there are two or more populations of receptors with different K_d values. (B) The Hill plot compares the log of the amount bound corrected for the maximal possible binding (B_{\max}) to the log of the ligand or transmitter concentration. The slope of this plot reveals if there is positive or negative cooperativity between the binding sites on a given receptor. If the slope is equal to 1, there is no cooperativity.

In the Scatchard plot, the amount of a radiolabeled transmitter or drug bound to its receptor is measured and plotted, as shown on the left side of Fig. 2. Briefly, the amount of a radioactive ligand that is bound to a membrane preparation (x -axis) is plotted against the ratio of the amount bound to free ligand present in the reaction (y -axis). If this relation is not linear, as shown by the dotted line, it indicates that more than one population of receptors is present with high and low dissociation constants, or that a receptor has multiple binding sites and that there is cooperativity between these binding sites. The x -intercept of this plot provides the number of receptors present in a particular tissue (B_{\max}) and the negative reciprocal of the slope gives the K_d of those receptors.

The Hill plot can be used to determine whether a receptor-agonist interaction displays positive or negative cooperativity. Cooperativity means that the binding of one transmitter molecule to a receptor influences the binding and action of another molecule of the same transmitter. The log of the ratio is the amount of a drug that is bound at a given dose of that drug over B_{\max} —the amount bound is plotted against the log of the dose (Fig. 2). This relationship generally produces a linear plot, and the slope of this line is usually referred to as the Hill coefficient, n_H . If the slope is 1, there is no cooperativity. If the slope is greater than 1, there is more than one binding site for the drug causing positive cooperative interaction. The point at

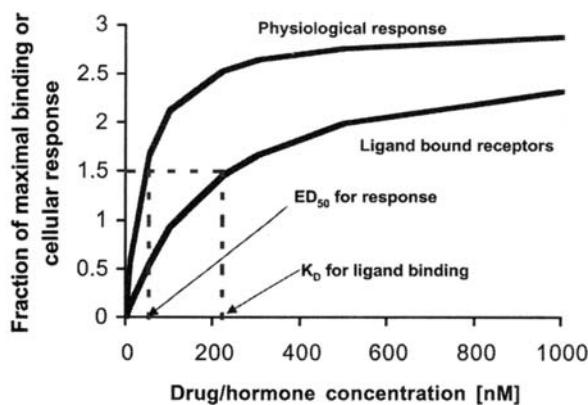


Fig. 3. Comparison of binding and response parameters typically observed in response to increasing amounts of a ligand (transmitter or drug agonist).

which the abscissa value equals zero also represents the apparent K_d for this binding interaction.

These same analytical approaches are applied to the characterization of dose-response relationships for drugs and transmitters in isolated tissue or *in vivo* but yield different parameters, E_{max} and ED_{50} for *in vivo* or EC_{50} for *in vitro* studies (Fig. 3). If the ED_{50} of the physiologic effect produced by a drug is compared with the K_d measured by the binding of this drug to its receptor, a discrepancy usually arises that results in the production of the maximal physiologic effect at a concentration well below that required to saturate the available receptor population in this tissue. This discrepancy led R.P. Stephenson to propose the concept of spare receptors—that in theory it is not necessary to occupy all of the available receptors to elicit the maximal response possible for a cell or tissue. Stephenson also attempted to explain differences in the ability of drugs to produce the same effect in a given tissue by proposing that the drugs had different efficacies. *Efficacy* is a pharmacologic term that refers to the intrinsic ability of a drug to produce a response.

2.2. Receptors Can Be Multisubunit Ion Channels in the Membrane or Single Polypeptides That Modulate an Effector System

Much of our current knowledge regarding the appearance of cellular receptors and how they work has come from studies in which the receptors were solubilized from the plasma membrane, chromatographically purified, and then reconstituted into lipid vesicles called liposomes. This work was tedious but crucial to our understanding of the receptor complex. The second major scientific approach that has helped

us understand receptors has been the molecular biological methods needed to “clone” receptor genes and sequence them to determine the amino acids of the expressed proteins. The use of “homology” cloning and sequencing (e.g., isolating unknown genes by using molecular probes for known genes or proteins) has revealed a wide variety of receptors, sometimes for totally unrelated transmitter systems. Such was the case for the muscarinic receptor, which was found to have a high degree of homology (e.g., degree of structural similarity) to the adrenergic-type, single-subunit receptors. Two receptors that received much of the early interest fall into each of the major classes of receptor families. The nicotinic acetylcholine receptor is a ligand-gated (e.g., directly triggered) ion-channel receptor type, and the β -adrenergic receptor is a G protein-coupled (e.g., second-messenger) receptor type.

2.3. Ligand-Gated Ion Channel Receptors

Ligand-gated ion-channel receptors are composed of four or five protein subunits, each of which has four membrane-spanning domains. The nicotinic cholinergic receptor was studied largely because of its accessibility. The electric organ of the eel and the ray is a tissue that is rich in cholinergic nicotinic receptors. Much effort has gone into isolating and characterizing the receptor from the membrane of this tissue. The isolated receptor appeared to be “oligomeric” because it had several protein subunits. The receptor subunits were isolated and purified, and part of the amino acid sequence was determined. The possible DNA sequences (e.g., DNA probe) coding part of the receptor protein were synthesized. Using these small DNA probes, known as oligoprobes, a library of genes was screened for homologous sequences. A messenger RNA was isolated and sequenced that was similar to the isolated protein. Surprisingly, many of the cloned subunits demonstrated a great deal of homology. This was particularly true in the hydrophobic segments that are believed to span the lipid membrane. These methods have confirmed that the nicotinic receptor contains several protein subunits that together surround an ionic channel through the membrane. These subunits exhibit many differences. An α subunit contains an extracellular sequence on the amino-terminal end that binds the transmitter acetylcholine. Two of these α subunits are contained in each of the muscle-type nicotinic receptors.

In all of the ligand-gated receptor proteins (e.g., nicotinic, γ -aminobutyric acid [GABA], glycine, and glutamate receptors), one of the membrane-spanning domains of each subunit contains a repeating sequence

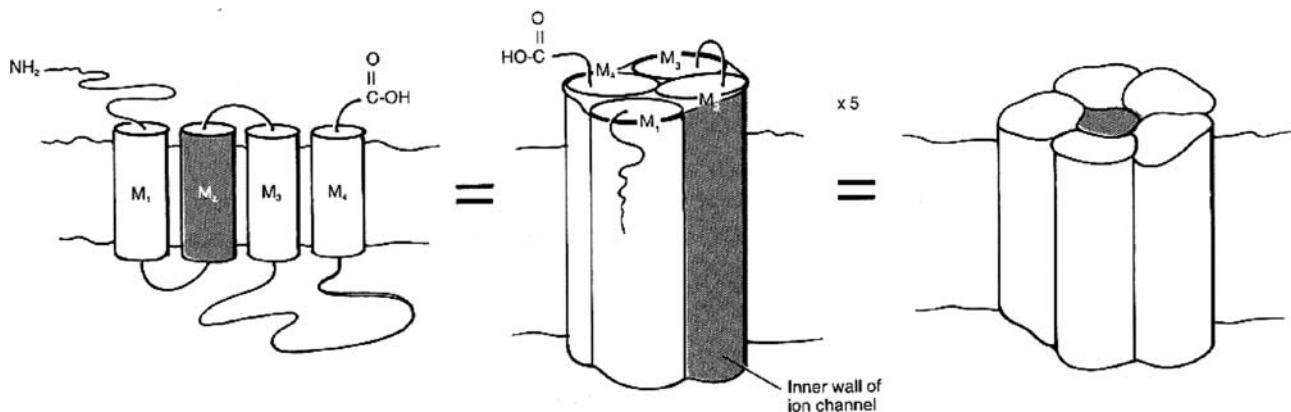


Fig. 4. The structural components of a ligand-gated ionic-channel receptor. The graph shows the subunit protein with the four membrane-spanning domains (*left*). This protein clusters to form a single subunit of the receptor (*center*). Five of these subunits fit together to form a ligand-gated ion channel (*right*). The M₂ subunit (*shaded*) has repeating sequences of charged amino acids and makes up the inner wall of the ionic channel.

of the polar amino acids, threonine, and serine. These are believed to line the channel surface itself and serve as the lipid-water interface within the channel. Each receptor has five of these channel-lining domains, α -helical sections known as the M₂ domains, one contributed by each of the five subunits. Our concept of the receptor subunits that combine to make up the pentameric nicotinic receptor is shown in Fig. 4.

Since the elucidation of the nicotinic-receptor structure, several other ligand-gated ionic-channel systems have been described. These include GABA, A-type and the glycine receptors, which form channels for chloride ion and function to hyperpolarize or inhibit nerve cells; several subtypes of the glutamate receptor that compose a channel for calcium, sodium, and potassium ions, similar to the nicotinic receptor; and the serotonin (5-HT₃) receptor that is an intrinsic channel for cations. Table 1 lists the known ligand-gated ion channels and some characteristics of these receptors. All of these receptors except the N-methyl-D-aspartate (NMDA) receptor are related in that they are pentameric, have four membrane-spanning domains per subunit, and have an M₂-spanning domain that lines the ion channel.

When the receptive subunit of these receptors is occupied by a neurotransmitter, a conformational change of the five-subunit complex allows the channel to open and the appropriately sized ion to enter the cell (Fig. 5). The type of ion that can traverse the channel seems to depend on the molecular radius of the ion, how tightly it combines with water molecules (free energy of hydration), and the strength of polar sites within or just outside the channel. For example, the sodium ion is smaller than the potassium ion but

Table 1
Attributes of Ligand-Gated Ion-Channel Receptors

Receptor	Subtype	Comment	Effector*
GABA	A		Cl ⁻
Glutamate	NMDA	Glycine [†]	Na ⁺ /K ⁺ /Ca ²⁺
	AMPA		Na ⁺ /K ⁺ /Ca ²⁺
	Kainate		Na ⁺ /K ⁺ /Ca ²⁺
Glycine		Strychnine [‡]	Cl ⁻
Serotonin	5-HT ₃		
Nicotinic	Muscle	50 ps [§]	Na ⁺ /K ⁺ /Ca ²⁺
	Neural	15–40 ps [§]	Na ⁺ /K ⁺ /Ca ²⁺

*The effector pathways represent the opening of ionic channels.

[†]The NMDA glutamate receptor has a glycine-binding site.

[‡]The glycine receptor is strychnine-sensitive.

[§]The muscle nicotinic receptor is open longer than is the neural receptor; time is given in picoseconds.

is excluded from the potassium channel. This is probably because it has a higher free energy of hydration and affinity for water molecules and cannot be dehydrated by the relatively weak ionic charges of the amino acids that make up the potassium channel. All of the cationic (e.g., Na⁺, K⁺, Ca²⁺) or positively charged ion channels have groupings of negatively charged amino acids (e.g., glutamate and aspartate) close to the mouth of the ion channel. These amino acids are probably important in removing the water from the cations (e.g., dehydrating) and producing Na⁺ or K⁺ channel selectivity.

In the case of the chloride channel for GABA or glycine, positively charged amino acids (e.g., arginine and lysine) are localized near the mouth of the channel. This is important in allowing the anions to enter

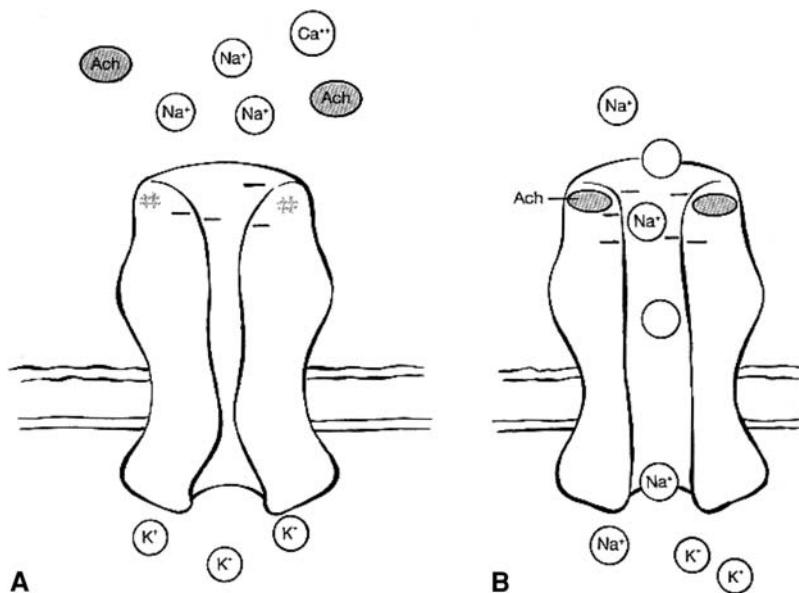


Fig. 5. (A) Resting and **(B)** stimulated nicotinic receptors. Combination of the α subunits of the receptor with acetylcholine causes the channel to open and allows sodium to enter the neuron and depolarize it. This receptor is drawn roughly to scale to show that about two-thirds of the receptor protein is extracellular.

the channel. The polar serine and threonine residues that line the M2 domain of the channels do not appear to determine the ionic specificity of the channel but are essential for maintaining the aqueous interface.

2.4. G Protein-Coupled Receptors

The G protein-coupled receptors (GPCRs) are composed of a single-polypeptide chain that has seven membrane-spanning domains. The prototypical G-protein receptor is represented by the β_1 -adrenergic receptor of the heart, which has received much attention because of the development of specific drugs that interact with it and the therapeutic importance of these drugs in treating heart disease, asthma, and hypertension. Studies of the cardiac β receptor indicate that it is associated with a membrane-bound protein that has guanylate nucleotidase activity, hydrolyzing GTP to guanosine 5' diphosphate (GDP). The β receptor was painstakingly solubilized from the membrane and partially sequenced. Eventually, this led to the cloning of a gene for a protein that had seven membrane-spanning domains. Figure 6 shows the current conception of the GPCR. The amino-terminal portion of the protein is found extracellularly, and the carboxyl-terminal end is inside the cell. There is a large intracellular loop of protein between spanning domains five and six. This loop is believed to interact with and regulate the G-protein "transducer" that is responsible for initiating the postsynaptic response.

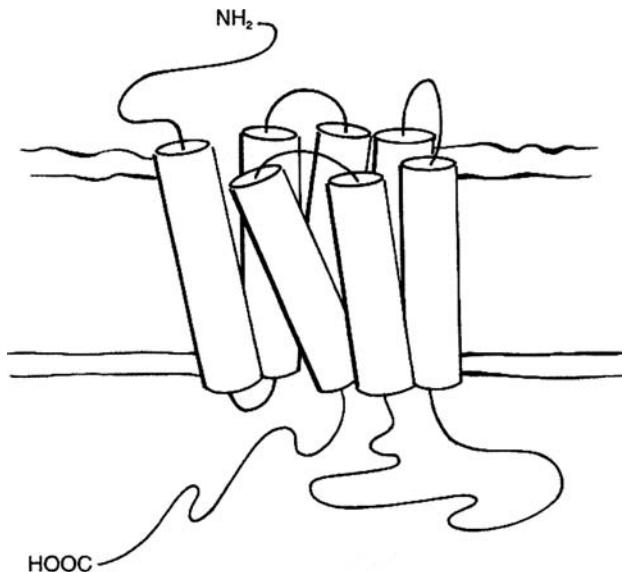


Fig. 6. The guanine nucleotide-binding regulatory G protein-coupled receptor protein (GPCR) is composed of seven hydrophobic membrane-spanning domains with three intracellular and extracellular loops of amino acids. The amino-terminal end of the protein is extracellular, and the carboxyl-terminal end is in the cytoplasm of the postsynaptic neuron. The third intracellular loop is believed to be responsible for activating the appropriate G protein.

Homology cloning of these GPCRs uncovered a wide variety of different receptors. Many of these are shown in Table 2. When genetic probes were made

Table 2
Characteristics of Selected G Protein–Coupled Receptors

Receptor type	Subtypes	AA/TMD*	Effector pathways†
Adenosine	A ₁ , A ₃ A _{2A} , A _{2B}	h326/7, h318/7 h412/7, h332/7	↓cAMP ↑cAMP
Adrenergic	α _{1A} , α _{1B} , α _{1D} α _{2A} , α _{2B} , α _{2C} β ₁ , β ₂ , β ₃	h466, h519, h572/7 h450, h450, h461/7 h477, h413, h408/7	IP ₃ /DAG ↓cAMP ↑cAMP
Cannabinoid	CB ₁ , CB ₂	h472, h360/7	↓cAMP
Dopamine	D ₁ , D ₅ D ₂ , D ₃ , D ₄	h446, h477/7 h443, h400, h387/7	↑cAMP ↓cAMP
Glutamate	mglu ₁ , mglu ₅ mglu ₂ , mglu ₃ , mglu ₄ , mglu ₆ , mglu ₇ , mglu ₈	h1194, h1212/7 h872, h877, h912, h877, h915, h908/7	IP ₃ /DAG ↓cAMP
Histamine	H ₁ H ₂ H ₃	h487/7 h359/7 h445/7	IP ₃ /DAG ↑cAMP ↓cAMP
Serotonin‡	5-HT _{1A} , 5-HT _{1B} , 5-HT _{1D} , 5-HT _{1E} , 5-HT _{1F} 5-HT _{2A} , 5-HT _{2B} , 5-HT _{2C} 5-HT ₄ , 5-HT ₆ , 5-HT ₇ 5-HT _{5A} , 5-HT _{5B}	h421, h390, h377, h365, h366/7 h471, h481, h458/7 h387, h440, h445/7 h357, m370/7	↓cAMP IP ₃ /DAG ↓cAMP ??
Muscarinic	M ₁ , M ₃ , M ₅ M ₂ , M ₄	h460, h590, h532/7 h466, h479/7	IP ₃ /DAG ↓cAMP

*Number of amino acids (AA) in the receptor/number of transmembrane-spanning domains (TMD). The letter before the number of amino acids denotes the species; h, human; m, mouse.

†The effector pathways produce changes in adenylate cyclase resulting in changes in cyclic 3'5'-AMP or modulation of phospholipase activity with changes in inositol trisphosphate (IP₃) and diacylglycerol (DAG) as second messengers.

‡The lower case (5-ht) denotes a cloned receptor with no physiologic correlate at present.

↑ = increase; ↓ = decrease.

against portions of the known G-protein receptors and compared with a gene library, several previously unrecognized genes for receptor subtypes were revealed. For example, five separate genes were identified for the muscarinic cholinergic receptor. Table 2 lists the most important functional effects of many GPCRs.

2.4.1. G PROTEINS

A major receptor-transducing element is called the guanine nucleotide (G) protein and is composed of three protein subunits. An integral part of this family of receptors is the G protein, which represents the transduction element between the receptor and the second-messenger system that ultimately induces the cellular response. Much of the functional specificity of these receptors resides in the type of G protein to which they preferentially couple. For example, the α₁- and α₂-adrenergic receptors respond to endogenous norepinephrine but initiate entirely different cellular events. The α₁-receptor activation activates a G protein (G_{q/H}),

which initiates the activity of membrane phospholipase, eventually resulting in increased intracellular calcium. The α₂ receptor, although similar in terms of the ligand-binding unit, activates what is called a G_i protein (i for inhibitory), which inhibits adenylate cyclase activity and generally causes inhibitory neuromodulatory activity. The β receptors couple to G_s proteins (s for stimulatory), which activate adenylate cyclase as well as many cellular processes. A similar picture has emerged for the families of dopamine and muscarinic cholinergic receptors.

Other G proteins are involved in sensory modulation. In the nasal mucosa, odorant molecules bind to GPCRs that activate a G protein, called G_{olf} for olfactory G protein. One of the earliest characterized G proteins was called transducin (G_t). This molecule is responsible for initiating the signal from stimulation of the rhodopsin molecule by light. Rhodopsin is a receptive protein with seven membrane-spanning domains that is covalently bound to 11-*cis*-retinal.

The G-protein complex is actually composed of three protein subunits known as the α , β , and γ subunits. There is an excess of α subunits in the cytoplasm that probably compete for the appropriate β and γ membrane-bound subunits. The β and γ subunits are highly conserved membrane proteins, and they anchor the α subunit near the receptor. The $\beta\gamma$ complex also stabilizes the binding of GDP to the α subunit and inhibits its activation. When the receptor is occupied by a transmitter, the α subunit is released and becomes activated. The α subunit is capable of binding GTP and activating or inhibiting the effector or second-messenger system, such as adenylate cyclase. The G-protein system allows for a high degree of amplification of the signal.

Many bacterial toxins interfere with G-protein systems. The agent responsible for cholera—cholera enterotoxin—irreversibly activates the G_s protein, increasing the intracellular cyclic AMP. Pertussis toxin irreversibly inhibits the G_i proteins.

2.4.2. EFFECTOR MECHANISMS

For many effector mechanisms, the final steps in the postsynaptic response include the activation of a second-messenger system by the α subunit of the G protein and triggering of a phosphorylation system. The phosphorylation of a protein induced by activating specific protein kinases results in the inhibition or, more often, activation of the phosphoprotein. Several second-messenger systems use this type of mechanism, including cyclic AMP, cyclic GMP, calcium-calmodulin, and the phospholipase C products, inositol triphosphate (IP_3) and diacylglycerol (DAG). Figure 7 shows

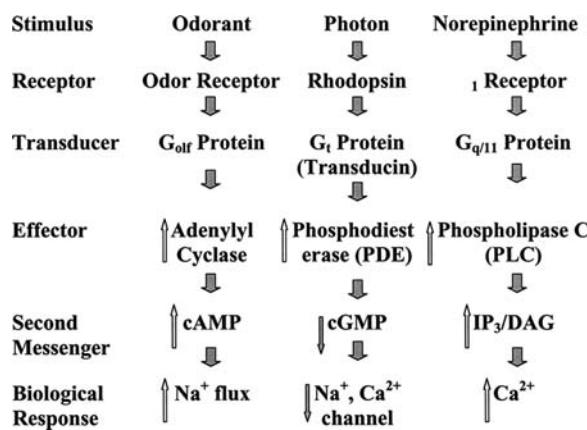


Fig. 7. Representative G protein-mediated response pathways for stimuli of olfactory, visual, and transmitter origin. PLC, phospholipase C; cAMP, cyclic 3'5'-adenosine monophosphate; cGMP, cyclic 3'5'-guanosine monophosphate; IP_3 , inositol trisphosphate; DAG, diacylglycerol. The arrows indicate activation (\uparrow) or inhibition (\downarrow).

several G-protein and second-messenger or effector pathways for sensory stimuli such as smell and vision and for neurotransmission by norepinephrine.

2.4.3. CONSTITUTIVE ACTIVITY AND INVERSE AGONISM

One of the most important recent advances in understanding GPCRs is the recognition that the basal state of a receptor is not necessarily “off” or inactive (i.e., some receptors are intrinsically active in the absence of any bound ligand). Whereas this concept was first articulated in describing the effects of benzodiazepines on the $GABA_A$ receptor, a ligand-gated ion channel, in the early 1980s, it was quickly discovered to apply to GPCRs as well. Traditionally conceptualized as light switches that can be turned on or off, we now must think of GPCRs as dimmer switches or rheostats, capable of existing in multiple states between “full on” and “full off.”

The implications of this discovery are far reaching. In addition to agonist and antagonist, the term *inverse agonist* must be added to the list of categories for describing the effect of ligand binding to a GPCR. An inverse agonist is defined as a compound that produces a biological effect that is the opposite of that produced by an agonist on a given receptor. For example, if epinephrine produces a transient increase in the level of cyclic adenosine monophosphate (cAMP) in a cell when binding to a β -adrenergic receptor, an inverse agonist binding to this receptor would produce a transient decrease in cAMP levels below the basal level. A reassessment of drugs formerly classed as antagonists now shows that many are actually inverse agonists.

2.4.4. RECEPTOR DYNAMICS: MEMBRANE TRAFFICKING

Although the capacity for neurons to alter the number of receptors expressed on their surface has long been recognized, only recently have we begun to understand the mechanisms by which this is accomplished. By modifying receptor number on the plasma membrane, a neuron alters the amplification or “gain” produced by a given signal in the form of neurotransmitter released or drug concentration. If the signal is too strong, receptors are initially uncoupled from the transduction mechanism in a reversible manner to limit the amplification produced. If the signal remains too strong for an extended time period, a process is initiated by which receptors are internalized rendering them unavailable for signal transduction. At this point, if the signal decreases, the process reverses and the internalized receptors return to the cell surface.

However, if the signal remains too strong, the internalized receptors are degraded by enzymatic action.

The uncoupling and internalization of GPCRs is apparently initiated by the activation of protein kinases that phosphorylate highly conserved sites on the intracellular domains of the receptors. Phosphorylation of these sites increases the affinity of the receptor for binding to additional proteins, such as β -arrestin, which facilitates binding of the receptor to clathrin to initiate internalization via clathrin-coated pits. In some cells, internalization appears to use a caveolin-mediated internalization mechanism rather than clathrin. Reversal of this process is mediated by phosphatases that remove phosphate groups from the proteins.

The dynamic interplay of kinases and phosphatases in regulating the phosphorylation state of GPCRs represents another important mechanism for modulating receptor function. With the introduction of these additional control points comes the opportunity for developing new pharmacologic approaches to altering synaptic activity and, unfortunately, yet another potential point for genetic mutations to produce disease states.

2.5. Nitric Oxide

A novel effector system that may also represent a transducer or a transmitter is the simple molecule nitric oxide. Although muscarinic vasodilation had been studied for decades, the exact mechanism of this action of acetylcholine was unknown. The muscarinic receptor in the vasculature is not innervated, but it can be activated by circulating choline esters. In the early 1980s, Furchtgott found that the muscarinic receptor of the vasculature occurred on the endothelium rather than vascular smooth muscle. The well-known vasodilation induced by stimulation of this receptor was found to be a secondary effect of the production of nitric oxide by the endothelium. This small, very short-lived molecule easily traverses lipid membranes, enters the smooth muscle, where it binds to and activates a soluble guanylate cyclase, which produces cGMP. In turn, cGMP activates a variety of proteins including myosin light-chain kinase, which act in concert to relax vascular smooth muscle. Nitrate vasodilators, such as nitroglycerin, have been used for years to alleviate angina pectoris. This is another example of the body having an endogenous chemical that resembles drugs that have been used for centuries.

Originally called endothelial-derived relaxing factor, nitric oxide is generated by the action of an enzyme that uses the amino acid arginine as a substrate. The enzyme is known as nitric oxide synthetase and is

widely distributed in the brain. In the future, it will be interesting to see whether this compound is given transmitter, transducer, or effector status in the overall scheme of how the brain works.

2.6. Receptors as Part of the Effector Pathway

Some receptors are part of a second messenger-generating system and directly stimulate the effector pathway. These receptors are themselves the transducers and the generators of the second messengers. Some of these are membrane-bound proteins that include receptors for growth factors, atrial natriuretic peptide, and activin. In the case of growth factors and the polypeptide activin, the receptors themselves are enzymes. Activation by these molecules causes receptor phosphorylation that allows them to function as a protein tyrosine kinase or protein serine-threonine kinase, respectively. The atrial natriuretic peptide receptor is a guanylate cyclase that results in increased intracellular cyclic GMP. Each of these membrane-bound receptors has only one membrane-spanning domain that anchors it to the membrane, unlike the seven spanning domains of the GPCRs. Others, such as the steroids, bind to soluble cytoplasmic receptors that interact with specific promoter regions of genes to initiate or inhibit gene translation. This group of receptors is not discussed in this chapter.

3. NEUROTRANSMITTERS AND THEIR RECEPTORS

Mushrooms, herbs, and bacterial and snake toxins have contributed much to our knowledge of neurotransmitters and their receptors. Early in the development of human civilization, pharmacology was mostly the domain of herbalists and alchemists. Plant and animal products have provided useful sources of drugs for various ailments and research tools to advance our knowledge of the function of the body and brain. Some of these remedies were quite sophisticated—the plant belladonna, which contains the alkaloid atropine, was burned in an open fire and inhaled by the ancient Hindus for allergies and asthma. Curare, the nicotinic blocker, from the Amazonian jungles, was used to paralyze game; and willow bark (e.g., salicylate) was used to cure fever and pain. Bacterial toxins from *Vibrio cholerae* and from *Bordetella pertussis*, which causes whooping cough, interact with G proteins. The snake toxin α -bungarotoxin was essential for characterizing and purifying the nicotinic receptor. These natural products have proved to be important for our current understanding of the brain transmitters and receptors that mediate bodily function and cognition.

3.1. Cholinergic Receptors Are Found in Both Main Genetic Families of Transmitter Receptors

Cholinergic receptors were the first receptor types to be identified and pharmacologically classified in the early 1900s. The subdivision of these receptors grew out of an interest in herbs that have been used for centuries as medicines or poisons. The major subdivisions of the cholinergic receptor systems are shown in Fig. 8 with a representative drug that selectively acts on that system.

3.1.1. NICOTINIC CHOLINERGIC RECEPTORS

Nicotinic receptors are ligand-gated ionic channels for sodium and selectively respond to the active ingredient in tobacco. The first major subdivision of the cholinergic receptor was named after a plant alkaloid, nicotine, which was introduced into western European cultures by Native Americans. Nicotine selectively stimulates cholinergic receptors at the neuromuscular junction and the autonomic ganglia. This receptor is also found in the brain, although it is much less prevalent than the muscarinic cholinergic receptor.

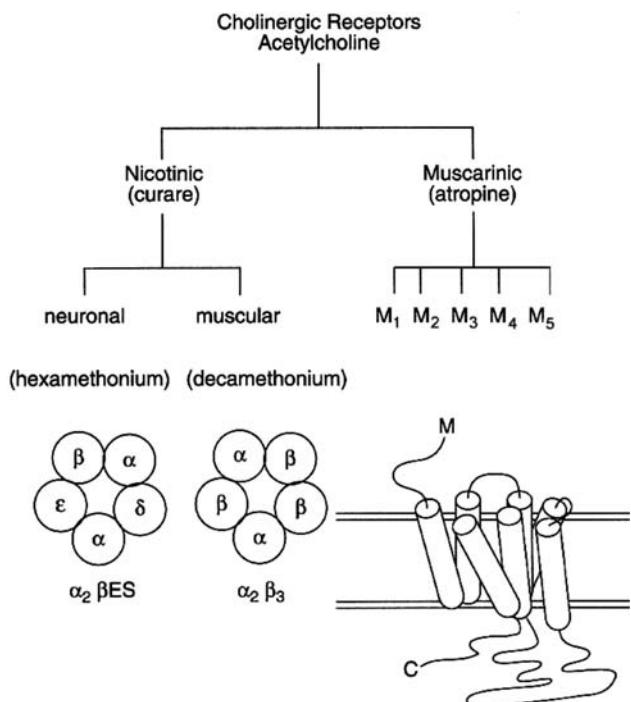


Fig. 8. The family of cholinergic receptors. This chart shows the subdivision of cholinergic receptors and the names of their subtypes. The drugs in parentheses are antagonists that block those receptors. The diagram below the chart shows the proposed subunit configuration of the two nicotinic receptors and the structure of the muscarinic receptor.

Early studies by Dale and Langley showed that, although semipurified acetylcholine stimulated skeletal muscle and the visceral smooth muscle, nicotine was selective only for the neuromuscular junction and the ganglia. Langley was able to map out the sympathetic and parasympathetic autonomic ganglia by applying a tincture of nicotine to peripheral-nerve ganglia and observing the visceral effects in the frog.

Further subdivisions of the nicotinic receptor were made during the 1930s and 1940s. Paton and Zaimis noticed that the nicotinic receptor of the neuromuscular junction was blocked by a molecule with two quaternary nitrogens separated by 10 carbon atoms (e.g., decamethonium) but that the autonomic ganglia was selectively blocked by a similar molecule with a separation of only six carbon atoms (e.g., hexamethonium). These molecules resembled two acetylcholine molecules linked back to back but separated by different distances. A second subdivision of the cholinergic receptor was then postulated: muscle and neuronal or ganglionic nicotinic receptors. Figure 9 shows the structures of hexamethonium, decamethonium, and acetylcholine and the subunit makeup of the two nicotinic receptors.

Much of the molecular biology of the nicotinic receptor was discussed in Section 2.3. The nicotinic receptor was originally isolated from the electric organ for the eel. To facilitate its purification, the snake-venom toxin, which tightly binds to the α subunit of the nicotine receptor, α -bungarotoxin, was used. By radiolabeling this toxin, the purification of the nicotinic receptor could be followed through various chromatographic steps.

With the help of molecular biologic techniques, we know that the human neuromuscular nicotinic receptor is very similar to the electric eel receptor. It consists of five subunits that compose a membrane channel that, when open, allows positively charged ions to flow into the cell. Two of the five protein subunits of the nicotinic receptor have binding sites for acetylcholine (i.e., α subunit). The makeup of the other three subunits may affect the distances between the α subunits of muscle-type and ganglia-type nicotinic receptors, which could explain the pharmacologic specificity of the receptors (Fig. 8). The neuronal or ganglionic receptor appears to have two α and three β subunits rather than the α (2), β (1), γ (1), and δ (1) makeup of the muscle receptor.

3.1.2. MUSCARINIC CHOLINERGIC RECEPTORS

Muscarinic cholinergic receptors are activated by muscarine, a substance found in the mushroom *Amanita muscaria*. Euripides, an ancient Greek, was the

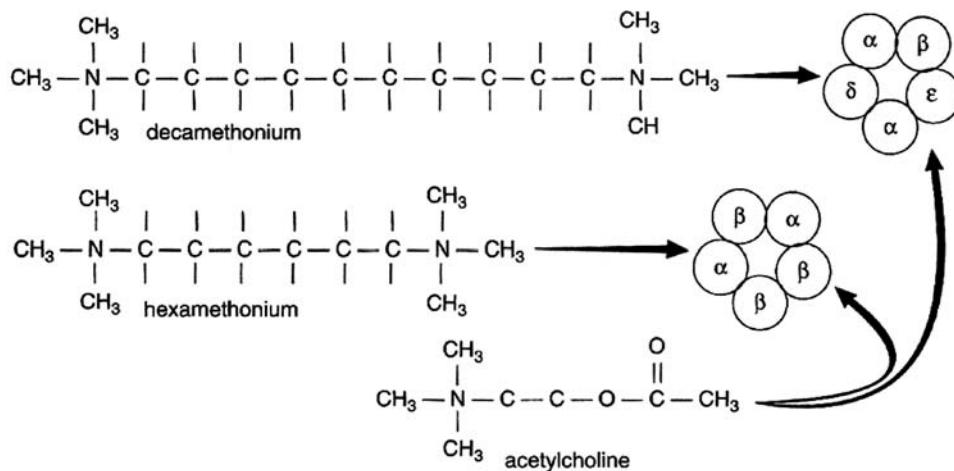


Fig. 9. Structures of selective neuromuscular and ganglionic (e.g., neural) nicotinic receptor antagonists. Decamethonium selectively blocks the muscle receptor, and hexamethonium blocks the neural receptor. The makeup of the pentameric subunits and the distance between the two subunits may partially explain this specificity. Acetylcholine stimulates both of the receptors.

first to describe the poisonous effects of *Amanita muscaria*, to which he lost several family members. The general syndrome of mushroom poisoning has become known as the SLUDE syndrome, which is an acronym for salivation, lacrimation, urination, defecation, and emesis. This acronym describes quite accurately the toxicologic effects of parasympathetic over stimulation by acetylcholine-like agonists, such as muscarine, and acetylcholinesterase inhibitors. This receptor is found on the postganglionic parasympathetic effector organs and in eccrine sweat glands. It is also the predominant cholinergic receptor found in the brain where it is 10 to 100 times more prevalent than nicotinic receptors.

How can the nicotinic and muscarinic receptors respond to the same neurotransmitter but also possess the capacity to respond selectively to different drugs? One explanation has been that acetylcholine is not a rigid molecule, because the two methyl groups separating the quaternary nitrogen of choline and the acetyl groups can freely rotate. This conformation allows the quaternary nitrogen and an atom capable of donating a pair of electrons to exist at various distances. When they are in their closest configuration (0.44 nm), they can “fit” into the muscarinic receptor, whereas when they are in their more distant configuration (0.59 nm), they are a better fit for the nicotinic receptor (Fig. 10). Examination of the structures of nicotine and muscarine has shown that these rigid molecules have chemical characteristics similar to acetylcholine and are separated by similar intermolecular distances.

Molecular biologic techniques have revealed other important differences between the nicotinic and

muscarinic receptor that explain their pharmacologic and physiologic uniqueness. Unlike the ligand-gated ion-channel nicotinic receptor, the muscarinic receptor is a GPCR receptor that uses a second-messenger system to accomplish its actions. Although acetylcholine is a fast transmitter (1 to 2 ms) when stimulating the nicotinic receptor, it acts like a slow transmitter (100 to 250 ms) when activating the muscarinic receptor. Like the β-adrenergic receptor, the muscarinic receptor is composed of a single membrane-bound protein that has seven membrane-spanning domains within its structure. Upon agonist activation, muscarinic receptors selectively bind and activate a G protein; subtypes M2 and M4 preferentially bind to G_{i/o} proteins, whereas subtypes M1, M3, and M5 preferentially bind to G_{q/11} proteins.

Five separate muscarinic-receptor genes have been cloned, each having unique distribution patterns and potentially distinct therapeutic importance. M1 muscarinic receptors are widely distributed in the brain, in the autonomic ganglia, and on hydrochloric acid-secreting cells of the stomach. This receptor activates the IP₃-DAG second messenger system via G_q protein activation and is selectively blocked by the drug pirenzepine. All muscarinic receptors are blocked by atropine.

The M2 receptor is found in the heart, hindbrain, bronchi, gastrointestinal tract, and bladder smooth muscle. Stimulation of this receptor leads primarily to the activation of K⁺ channels, but in some tissues, it decreases cellular cyclic AMP levels. In the smooth muscle of the bronchi and gastrointestinal tract, the G_i-mediated decrease in cyclic AMP will lead to

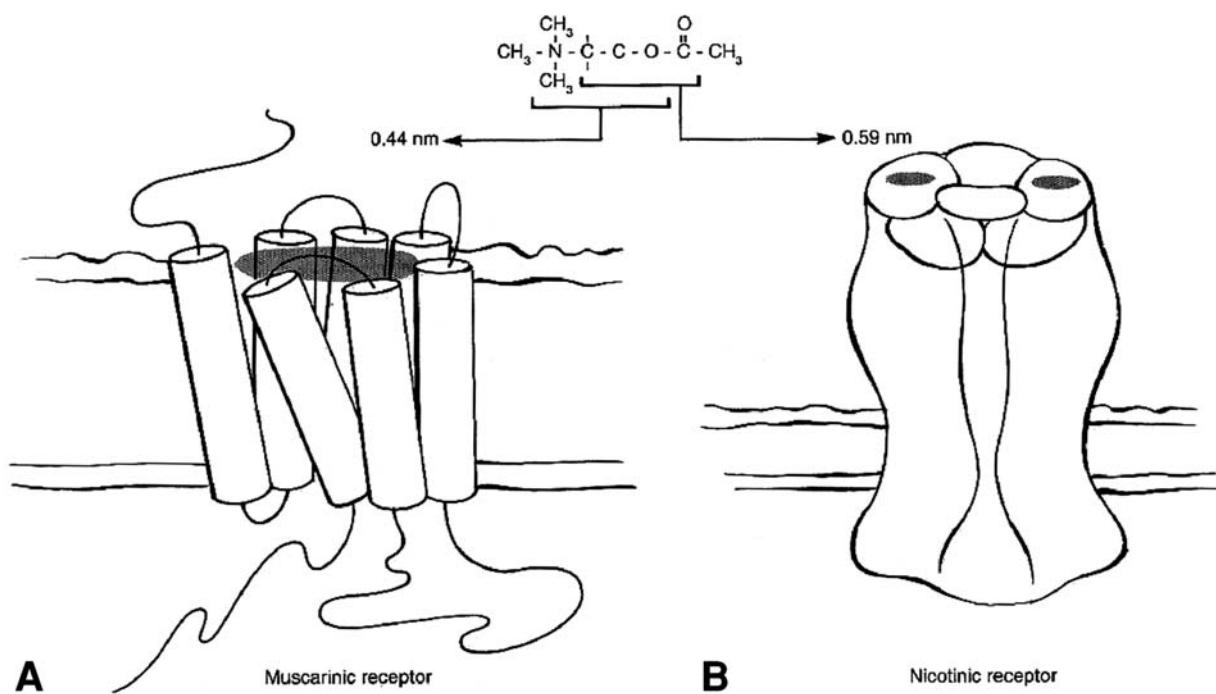


Fig. 10. Diagram of how acetylcholine can interact with the (A) muscarinic and (B) nicotinic receptors. This theory contends that the distance between the quaternary nitrogen of acetylcholine and an atom capable of donating a pair of electrons is the structural requirement for an agonist. Because acetylcholine has two methyl groups separating the nitrogen and the oxygen, it can exist in both configurations. The muscarinic receptor requires a distance of about 0.44 nm, and the nicotinic receptor requires 0.59 nm.

contraction. In the heart, activation of non-ligand-gated potassium channels decreases the heart rate and automaticity. In the brain, M₂ receptors are primarily located presynaptically and function to decrease neurotransmitter release.

M₃ receptors are found peripherally in glandular tissue and smooth muscle, whereas in the brain they are usually located presynaptically, like M₂ receptors, where they also function to decrease neurotransmitter release. M₃ receptors are particularly important for regulating salivary gland function and urinary bladder contractility. M₄ and M₅ receptors appear to be expressed exclusively within the brain in the striatum (M₄), substantia nigra (M₅), ventral tegmental area (M₅), and the cerebral vascular endothelium (M₅). Functionally, these receptors play an important role in modulating dopamine release affecting basal locomotor activity and reward pathways associated with the striatal and mesolimbic dopamine pathways, respectively.

3.2. Catecholamine Receptors

Soon after the discovery of sympathetic transmitters in the early 1900s, it was observed that the postsynaptic actions they produced were extremely variable, stimulating some systems and inhibiting others.

Specifically, they stimulated the heart and most vascular smooth muscle, but they inhibited bronchial, some vascular, and gastrointestinal smooth muscle. To explain these differences, Cannon proposed in the early 1920s that there were two sympathetic transmitters: sympathin E excited smooth muscle, and sympathin I inhibited smooth muscle. With the discovery of epinephrine in the adrenal glands and norepinephrine in the sympathetic nerves, this hypothesis held for decades. In 1948, Raymond Alquist proposed that the diversity of physiologic actions of catecholamines could be explained by the existence of distinctly different receptor subtypes. He called these adrenergic receptors the α and β receptors.

Alquist's hypothesis was based on the actions of several adrenergic agents and derivatives on visceral activity; among these agents were norepinephrine, epinephrine, and isoproterenol. The excitatory actions of the catecholamines were ascribed to α -receptor activation. The agents that were most potent in stimulating these actions were norepinephrine and epinephrine. α -Adrenergic activation by these agents included vasoconstriction and pupillary dilation. β -receptor stimulation tended to be predominately inhibitory and included bronchial dilation, decreased gastrointestinal

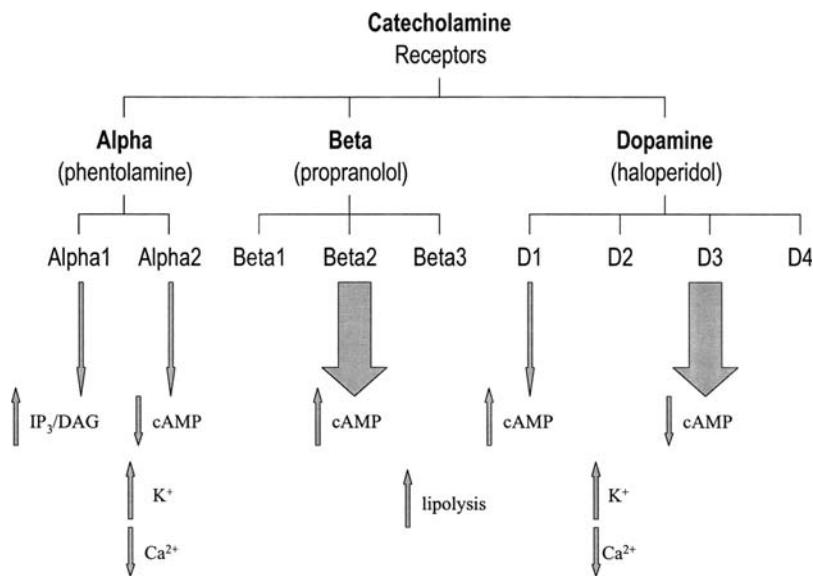


Fig. 11. The family of catecholamine receptors showing the subtypes and major effector pathways. The drugs in parentheses are selective blockers for the different receptors.

motility and bladder smooth-muscle tone, vasodilation of some vascular beds, and cardio-acceleration. The agents that stimulated these β -receptor functions tended to have larger substitutions on the amine group of the catecholamine. Isoproterenol and epinephrine were much more potent stimulators of these receptors than was norepinephrine, which has no methyl groups on the amine terminal. Many of the β -receptor agonists and antagonists synthesized subsequently share this property, strengthening the hypothesis that β -receptor selectivity is largely based on a bulky amino-terminal substitution of the biogenic amine. Figure 11 provides a breakdown of the various therapeutically important subtypes of adrenergic receptors.

3.2.1. α -ADRENERGIC RECEPTORS

α -Adrenergic receptors can stimulate postsynaptic events (α_1) and through presynaptic receptors can inhibit neuronal activity (α_2). The α -adrenergic receptors were pharmacologically subdivided in the 1970s, when the actions of some drugs were found to interact with presynaptic alpha-like receptors that inhibited neuronal activity. Molecular biological techniques have shown that there are six separate genes for various α receptors. All of these receptors and all of the known β and dopamine receptors belong to the G protein-coupled family, and each is a single membrane-bound protein that has seven membrane-spanning domains. Figure 11 describes the shared molecular mechanisms of many of the adrenergic receptors.

The α_1 receptor, as described by Alquist, is an excitatory receptor that couples to a G protein that activates the enzyme phospholipase C. This enzyme cleaves the membrane lipid, phosphatidylinositol bisphosphate (PIP_2), into two separate second messengers. DAG remains within the membrane and activates protein kinase C (PKC). This kinase is capable of phosphorylating serine and threonine residues of a number of substrates within the membrane or in the cytoplasm. The other second messenger derived from PIP_2 is inositol trisphosphate (IP_3). This water-soluble molecule is released into the cytoplasm, where it binds to IP_3 -sensitive Ca^{2+} channels on the endoplasmic reticulum (ER) to trigger the release of Ca^{2+} . Ca^{2+} then activates tissue-specific secondary-effector systems. The ability of the α_1 receptor to stimulate a single-transducer system to activate two second-messenger systems (PKC and cytosolic Ca^{2+}) from the same substrate (PIP_2) provides additional amplification of the original transmitter and α -receptor interaction.

The elucidation of the α_2 -adrenergic receptor helped to explain several confusing observations related to the autonomic nervous system and the central nervous system (CNS). Many of the side effects of α -receptor blockade seemed to resemble activation of the sympathetic and the parasympathetic nervous system. For example, the side effects of the nonselective α -blocking drug phentolamine included increased gastrointestinal motility (e.g., diarrhea) and elevated circulating

levels of the sympathetic transmitter norepinephrine. This plasma norepinephrine is able to activate the unblocked β receptors. With the development of α_2 -type agonists, such as clonidine, the opposite effects were found. This type of drug seemed to turn off sympathetic and parasympathetic activity. Clonidine is useful for high blood pressure because it decreases sympathetic norepinephrine release. However, one of its side effects is decreased gastrointestinal motility.

The α_2 -type receptors are found predominately on neurons within the brain, or presynaptically on sympathetic and parasympathetic nerves. The α_2 receptor is coupled to a G_i protein, which results in a decrease in cyclic AMP, inhibition of Ca^{2+} -channel opening, and increased potassium-channel activity (Fig. 11). The resulting effect is inhibition of neuronal activity. α_2 -Adrenergic agonists have been found to be useful for opiate and nicotine withdrawal symptoms.

3.2.2. β -ADRENERGIC RECEPTORS

Both of the major β -adrenergic receptors are coupled to a G_s protein, which results in an increase in cyclic AMP. β -Adrenergic receptor responses in the peripheral autonomic nervous system include dilation of a variety of smooth muscles, stimulation of metabolic functions such as lipolysis and glycogenolysis, and an increase in heart rate. From a therapeutic standpoint, drugs that are selective for the β receptors of different tissues are important. For example, a drug that selectively stimulates the β receptor of bronchial smooth muscle (causing bronchodilation) could be useful in asthma, and a drug that selectively blocks the cardiac β receptor might be beneficial for angina or high blood pressure. The search for selective β -adrenergic drugs did lead to the discovery of specific β_1 blockers (e.g., atenolol) and β_2 agonists (e.g., terbutaline) and the cloning of three different β receptors that range in size from 400 to 477 amino acids. All of these β receptors use the same transducer and effector systems. As shown in Fig. 12, they activate a G_s protein that stimulates cyclic AMP formation, leading to phosphorylation of various cellular serine and threonine residues by a cyclic AMP-dependent protein kinase.

The β receptor is the prototypical GPCR that was the first to be isolated and cloned (Fig. 13). The β_1 -adrenergic receptor is the subtype responsible for increased heart rate and lipolysis, and most of the other receptors, including those involved in bronchodilation, are defined as β_2 . (This can be easily remembered, because there is one heart [β_1] and only two lungs [β_2]). There are also β receptors in

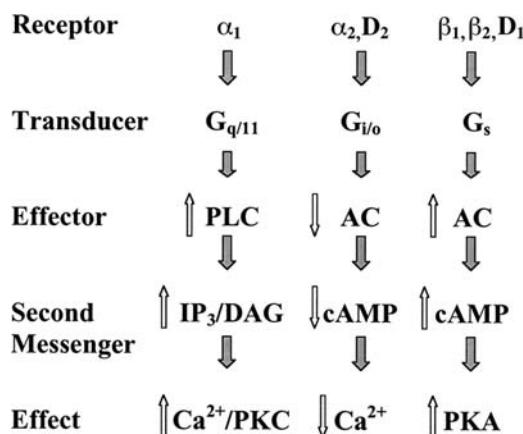


Fig. 12. Catecholamine effector pathways. Some G protein and effector pathway mechanisms are shared by different types of catecholamine receptors. PLC, phospholipase C; IP₃, inositol trisphosphate; DAG, diacylglycerol; PKA, protein kinase A; PKC, protein kinase C; AC, adenylate cyclase. The arrows indicate activation (\uparrow) or inhibition (\downarrow).

the CNS, although the subclassifications have not been worked out.

3.3. Dopamine Receptors

Central dopamine receptors are involved in the neuropathologies that underlie movement disorders such as Parkinson's disease and mental illness. Four dopamine receptors have been pharmacologically defined, and five have been cloned. Most of the actions of dopamine receptors occur in the CNS, although some do mediate peripheral functions. The central actions include vasodilation in the kidney vasculature (D_1 receptor), inhibition of sympathetic ganglionic activity (D_2), and inhibition of prolactin secretion by the anterior lobe of the pituitary (D_2). The D_2 receptor also inhibits acetylcholine release from the striatum. This is the transmitter function that is deranged in Parkinson's disease, and demonstrates why L-DOPA, the precursor of dopamine, and trihexyphenidyl, the cholinergic muscarinic blocker, are effective in alleviating the tremors associated with this disorder. Antipsychotic drugs, such as chlorpromazine and haloperidol, produce their therapeutic effects primarily through binding to D_2 receptors in the CNS. The D_1 and D_5 receptors stimulate cyclic AMP formation, and the D_2 , D_3 , and D_4 receptors inhibit the formation of this second messenger. These receptors are also capable of altering phosphoinositide turnover, inhibiting Ca^{2+} channels, and enhancing K⁺ conductance in some brain regions.

The dopamine D_2 receptor gene was the first to be cloned using a probe against the β -adrenergic receptor.

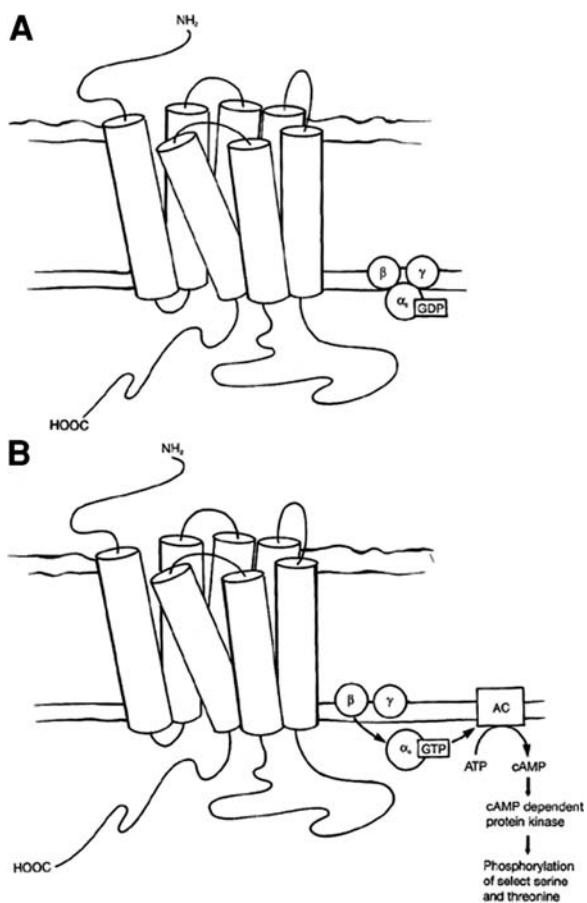


Fig. 13. Conceptualized G protein–coupled receptor. The β -adrenergic receptor is coupled to the G-protein complex. (A) The G protein is composed of β and γ subunits, which are anchored in the membrane, and an α subunit, which binds GTP and can be released into the cytoplasm to activate the second-messenger system. (B) In this case, the second messenger is cAMP, which is generated when adenylate cyclase (AC) is activated by the G protein.

The D₁ and D₂ receptors are both found in the caudate/putamen, nucleus accumbens, and olfactory tubercle. In contrast, D₃ receptors are found in the olfactory tubercle, nucleus accumbens, and the islands of Calleja, and the D₄ receptors are found in the frontal cortex, the midbrain, and the amygdala. D₅ receptors are expressed in the hippocampus, thalamus, and hypothalamus. The structures of the D₁ and D₂ receptors are shown in Fig. 14. The major differences are found in the size of the cytoplasmic loop between the membrane-spanning domains 5 and 6 and the size of the cytoplasmic carboxyl terminal. The D₂ receptor that inhibits adenylate cyclase has a long loop between the transmembrane (TM)-spanning domains 5 and 6 and a short carboxyl terminal. The opposite is true for the D₁ receptor. These cytoplasmic, intracellular loops of the protein are

believed to interact with their specific G protein to mediate the appropriate effector mechanisms.

3.4. Serotonin Receptors

Serotonin receptors are a diverse family in structure and function, including 13 G protein–coupled subtypes and one multisubunit, ligand-gated ion channel. Figure 15 shows the cloned serotonin receptors and their second-messenger systems. The GPCR subtypes can be grouped in families on the basis of which G proteins they preferentially interact with: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1E}, 5-HT_{1D}, and 5-HT_{1F} couple to a G_{i/o} transducer and inhibit cyclic AMP production; 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} couple to G_{q/11} and stimulate phosphoinositide turnover; 5-HT₄, 5-HT₆, and 5-HT₇ couple to G_s and stimulate cyclic AMP production. These receptors are important in sleep-wake cycles, cognitive function, and mental health problems such as schizophrenia and depression.

Only a small percentage of the total body serotonin is found in the brain; high concentrations are found in the periphery, particularly in the gastrointestinal tract and platelets. Nonetheless, brain serotonin is widely distributed and serves a wide range of functions. Physiologic studies have shown that the 5-HT₁ receptors tend to be inhibitory, and 5-HT₂ receptors are excitatory. The 5-HT_{1A} receptor is found in highest concentrations in the hippocampus and the raphe nucleus, where it appears to be an autoreceptor and inhibits the intrinsic pacemaker activity of the serotonergic neurons. The 5-HT_{1D} receptor also inhibits cyclic AMP formation and is most prevalent in the basal ganglia, globus pallidus, and the substantia nigra, where it may be involved in control of voluntary muscle. The 5-HT₂ receptor activates phosphoinositol metabolism and causes depolarization of neurons in the cortex. All of these 5-HT receptors have extensive homology with the other GPCRs. They show the greatest amount of variability in the third cytoplasmic loop, between the fifth and sixth membrane-spanning domains, which is an area believed to initiate the G-protein response.

The 5-HT₃ receptor is a ligand-gated ion channel that causes depolarization and excitation of neurons in the peripheral nervous system, entorhinal cortex, and the area postrema. Like other ligand-gated channels, the 5HT₃ receptor is composed of five protein subunits each with four transmembrane domains that coalesce to form an ionic channel.

3.5. Histamine Receptors

Specific histamine receptor–blocking drugs are therapeutically useful in motion sickness, as sedatives, and

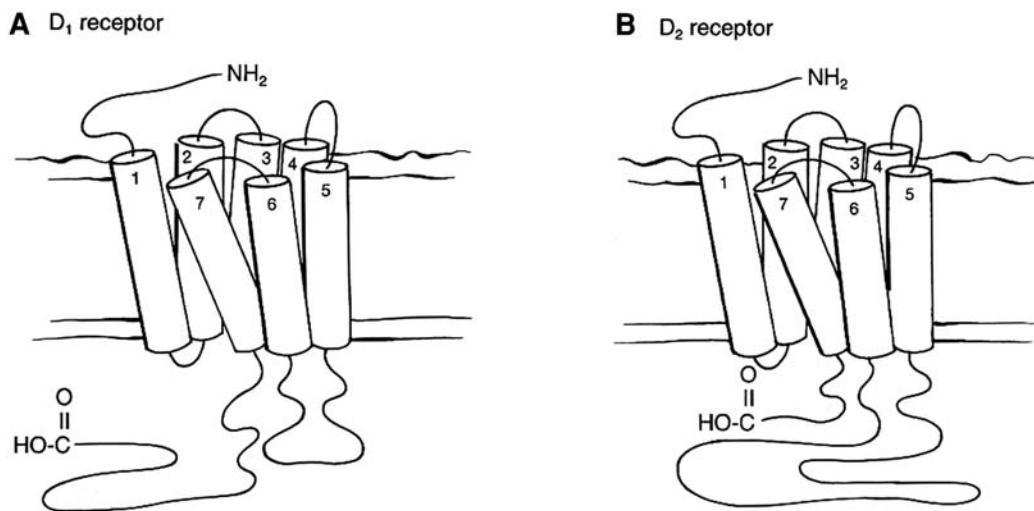


Fig. 14. The dopamine receptor family subtypes are drawn roughly to scale, showing the structure of the two dopamine (D1 and D2) receptors. **(A)** The D1 receptor stimulates adenylate cyclase and has a short third cytoplasmic loop between transmembrane domains 5 and 6. It also has a longer carboxyl-terminal end. **(B)** The D2 receptor, which inhibits adenylate cyclase, has a long third cytoplasmic loop and short carboxyl-terminal end. The seven membrane-spanning domains exhibit a high degree of homology.

for allergic disorders. Receptor binding and molecular biologic techniques have demonstrated the existence of three histamine receptors that are each coupled to a different type of G protein and therefore to completely different effector pathways. As is the case with most transmitters, histamine has peripheral and central actions.

When acting through H₁ receptors, histamine is a potent vasodilator, causing a drop in blood pressure with flushing of the face and engorgement of mucosal vasculature. This receptor is coupled to phosphoinositol metabolism, causing an increase in the second messengers IP₃ and DAG. The classic antihistamine drugs, such as diphenhydramine, work on this receptor. Blockade of histamine H₁ receptors in the brain causes sedation and inhibits motion sickness, which is

the basis of a large market for over-the-counter antihistamines. There is also evidence that the histaminergic pathway activates phospholipase A, which cleaves arachidonic acid from membrane phospholipids, activating lipoxygenase and cyclooxygenase pathways.

The histamine H₂ receptor increases cyclic AMP and is the target of drugs used to decrease gastric acidity. In the stomach, an H₂ receptor is responsible for stimulating the parietal cell to secrete hydrochloric acid. The selective H₂ blockers, such as ranitidine, block this action of histamine and have revolutionized the treatment of peptic ulcers. In the heart, there is an H₂ receptor that causes an increased heart rate that is not blocked by the β-blocking agent propranolol.

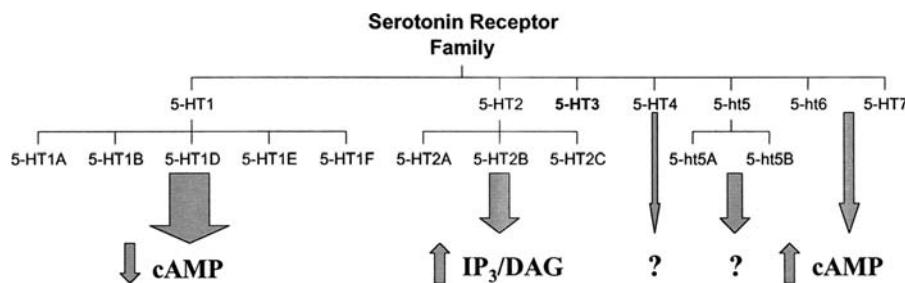


Fig. 15. The serotonin (5-HT) family of receptors showing the breakdown of receptor subtypes and their effector pathways. Notice that the 5-HT₃ receptor is a ligand-gated ion channel, whereas the rest are G protein-coupled receptors. IP₃, inositol trisphosphate; DAG, diacylglycerol. The effector pathways for the 5-HT₄ and 5-htr5 subtypes are currently unknown. The lower case (5-htr) denotes a cloned receptor with no physiologic correlate at present.

The H₃ receptor is widely distributed throughout the CNS and appears to function as an autoreceptor on many neurons. (An autoreceptor is defined as a cell-surface receptor that is sensitive to the neurotransmitter release by the neuron expressing this receptor.) In humans, the H₃ receptor has been shown to modulate the release of histamine and norepinephrine in the cerebral cortex, and it has been implicated in regulating the release of many additional neurotransmitters in the striatum, hypothalamus, hippocampus, substantia nigra, and spinal cord of the rat and other mammals. A variety of H₃-selective agonists and antagonists is now being evaluated for therapeutic potential for a number of disorders including Alzheimer's disease, attention-deficit hyperactivity disorder (ADHD), epilepsy, and obesity.

3.6. Receptors for Amino Acid Neurotransmitters

The amino acids glutamate, glycine, and GABA occur in concentrations 1000 times higher than those of the better-characterized biogenic amines and acetylcholine. The fundamental importance of these transmitters in CNS function appears to be reflected in the number and complexity of receptors that have been identified and their widespread distribution. Amino acid receptors fall into the two major receptor structural categories: GPCRs and ligand-gated ion channels.

3.6.1. GABA RECEPTORS

Both classes of GABA receptors are inhibitory, acting as ligand-gated chloride channels (GABA_A) or by modifying cyclic AMP and changing potassium and calcium channels (GABA_B). GABA serves as the major inhibitory transmitter in the brain and is usually found within interneurons in the cortex and cerebellum. (These neurons have relatively short axons.) The GABA_A receptor is a pentameric complex containing three or four different types of protein subunits (Fig. 16). All of the subunits have four TM domains and, like the glycine receptor, have extensive homology with the nicotinic cholinergic receptor. Many subunits have been cloned, including six α , four β , three γ , three ρ , and one δ , ϵ , π , and θ . Although the number of possible combinations of these subunits offers a mind-boggling array of putative GABA receptors, the most common subunit stoichiometry observed contains α , β , and γ subunits. The α and β subunits contribute to the GABA recognition site, and it appears that the α and γ subunits are necessary for benzodiazepine binding.

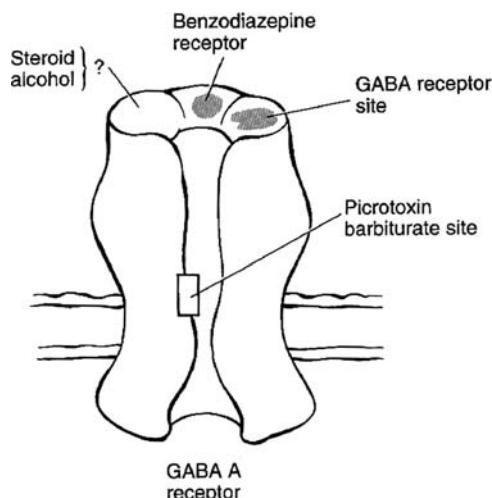


Fig. 16. Diagram of the GABA_A receptor. The GABA_A receptor is a pentamer of nonidentical subunits that form a chloride ion channel. It has specific binding sites for barbiturates, benzodiazepines, and steroid anesthetics, which augment the inhibitory action of GABA.

The GABA_A receptor is pharmacologically interesting, because it has specific binding sites for a number of seemingly unrelated drugs and agents. Benzodiazepine binding facilitates the inhibitory action of GABA on the receptor by augmenting the chloride-channel conductance. This explains much of the sedative and antianxiety action of benzodiazepines such as diazepam (Valium) or chlordiazepoxide (Librium). The ability of the GABA receptor to bind to benzodiazepines, as determined by affinity chromatography, was used to isolate and characterize these receptors. Other binding sites on the GABA_A receptor exist for the barbiturates, alcohol, and certain anesthetic steroids. All of these agents augment the action of GABA and inhibit CNS activity to induce sedation and sleep.

The GABA_B receptor is a GPCR and therefore is structurally and pharmacologically distinct from the GABA_A receptor. The GABA_B receptor was the first member of the GPCR family shown to form a heterodimer held together by coiled-coil interactions in the C-terminus and probably other domains. The receptor is activated when GABA binds to the N-terminus of one subunit (GABA_{B1}) producing a conformational change in the other subunit (GABA_{B2}). The activated receptor then interacts with G protein to initiate signaling. Heterodimerization has now been shown to occur between many other GPCRs and is believed to constitute an important functional interaction that contributes to the dynamic regulation of these receptors. Pharmacologically, the GABA_B receptor is selectively activated by baclofen, a drug that is used clinically to treat spasticity.

3.6.2. GLYCINE RECEPTOR

The glycine receptor is found in the spinal cord and brain stem and is inhibited by the poison strychnine. Although the GABA receptor is the principal inhibitory receptor in the brain and brain stem, the glycine receptor serves this role in the spinal cord. The inhibitory glycine receptor is a pentameric ligand-gated receptor composed of α and β subunits that form a chloride channel. Activation of this channel leads to the entry of chloride ion into the cell, hyperpolarizing the membrane and inhibiting neural activity. One of the best-understood glycine pathways in the spinal cord runs through the Renshaw cell, which inhibits the α motor neuron. When this important feedback inhibition is blocked by strychnine, the resulting seizures can lead to death. This is the basis for using strychnine in rat poison. Sensory information in the spinal cord is also probably filtered through glycine receptors, because low doses of strychnine increase tactile sensations. Although strychnine has a long history of use, there is no rational therapeutic basis for its use.

Glycine plays an important modulatory role through a binding site on one of the glutamate receptors. Glycine binding to this NMDA subtype of the glutamate receptor increases the frequency of cation-channel opening (e.g., excitation). This effect is not antagonized by strychnine.

3.6.3. GLUTAMATE RECEPTORS

The acidic amino acids glutamate and aspartate stimulate almost all brain neurons. This action is so widespread in the CNS, in contrast with the discrete sites of action for other transmitters, that it was assumed for many years to be a nonphysiologic, insignificant response to a metabolic intermediate. However, as experimental results mounted that satisfied more of the criteria for a neurotransmitter, acceptance of the transmitter role for these compounds was eventually achieved. Furthermore, at least 11 different excitatory amino acid receptors are now known to exist, and specific neural pathways that use these acidic amino acids as their transmitters have been defined. Glutamate appears to be the major stimulatory transmitter system in the brain. These pathways seem to have long axonal projections and communicate information between major brain structures, such as the cortex to midbrain areas.

The 11 known glutamate-receptor subtypes belong to both the ligand-gated ion-channel family ("ionotropic": NMDA, AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), kainate subtypes), and the GPCR family ("metabotropic" glutamate receptors:

mglu₁₋₈). The ionotropic glutamate receptors seem to belong to a different gene family than do the nicotinic, GABA, and glycine receptors. In the glutamate receptors that have been cloned, the extracellular amino terminal of the subunit peptides is much longer, containing almost 500 amino acids rather than the 200 found in the nicotinic receptor. Like the other ligand-gated channels, the non-NMDA glutamate receptors are pentameric and have four TM domains within each subunit; the subunit stoichiometry of the NMDA receptor remains unknown (Fig. 17).

Activation of an ionotropic glutamate receptor by an excitatory amino acid opens a cationic channel that depolarizes and stimulates the postsynaptic neuron. In the case of the AMPA- and kainate-receptor subtypes, stimulation by the drugs quisqualate and kainate, respectively, quickly renders the channel permeable to sodium and potassium. The NMDA receptor is relatively slow and has an unusual form of voltage

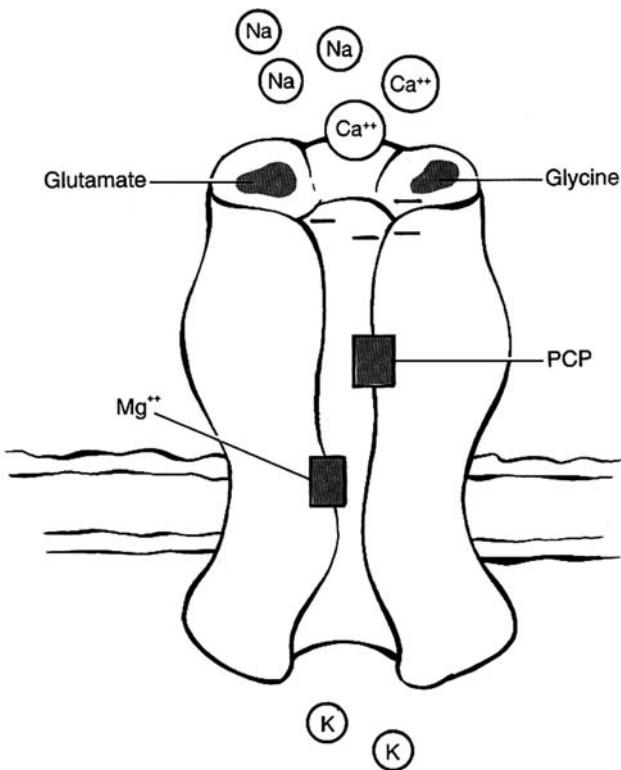


Fig. 17. NMDA subtype of the glutamate receptor. The diagram of the glutamate *N*-methyl-D-aspartate (NMDA) receptor shows ligand-binding sites for glutamate, glycine, magnesium, and the drug phencyclidine (PCP). When the cell becomes depolarized, magnesium is released from the interior of the channel, which allows glutamate to open the receptor channel. A glycine-binding site is also needed for channel activation. The NMDA receptor is unique in that calcium can enter the channel, as do sodium and potassium.

regulation. The channel has a Mg²⁺-binding site that inhibits ion flow when occupied. Mg²⁺ binding to this site is voltage-dependent, so that when the neuron is depolarized by other neural mechanisms, the Mg²⁺ can be displaced and the receptor channel opened by glutamate or NMDA. The NMDA-receptor channel is permeable to Ca²⁺ in addition to sodium and potassium. A unique aspect of the NMDA receptor is that it requires the presence of glycine to function adequately.

The NMDA receptor is distributed throughout the brain, particularly in the cortex and hippocampus. NMDA receptors appear to play an important part in the development of long-term depression (LTD), long-term potentiation (LTP), and synaptic plasticity. (LTD and LTP represent cellular forms of “memory.”) The distribution of the AMPA receptor parallels that of the NMDA receptor, and the kainate receptor is localized in specific regions of the brain, including the hippocampus. Overactivation by glutamate and the application of large concentrations of the selective agonists (e.g., kainate and ibotenic acid) is neurotoxic. It appears that overstimulation of these ligand-gated glutamate channels triggers postsynaptic neuronal death, probably by elevating intracellular Ca²⁺ to toxic levels.

The metabotropic glutamate receptors are currently divided into three groups based on similarities in primary sequence, agonist pharmacology, and G protein-effector coupling. Group I receptors (mglu₁ and mglu₅) preferentially couple to G_{q/11}, which links them to

phospholipase C and the production of DAG and IP₃. Group II receptors (mglu₂ and mglu₃) have very similar pharmacologic profiles and couple to G_{i/o} to inhibit cyclic adenosine monophosphate (cAMP) production. Group III receptors (mglu₄, mglu₆, mglu₇, and mglu₈) also couple to G_{i/o} but are pharmacologically distinct from group II. Structurally, these receptors differ from most GPCRs in that they have a large N-terminal domain, which serves as the glutamate-binding site. Although metabotropic glutamate receptors are widely expressed throughout the brain, specific subtypes appear to be differentially expressed. As a class, the metabotropic glutamate receptors appear to play an important neuromodulatory role in the CNS.

3.7. Peptide Receptors

Just as there was an explosion in the discovery of neuroactive peptides around 1970, the advent of molecular biologic techniques stimulated the discovery of a vast array of neuropeptide receptors. Almost all of these belong to the GPCR family, but the exceptions are important. Atrial natriuretic peptide is also present in the brain and acts on a protein receptor that has only one membrane-spanning domain. The cytoplasmic extension of this receptor is itself a guanylate cyclase, which is activated when the receptor is occupied by an agonist. Table 3 lists representative neural peptides, their subtypes, their effector pathways, and the number of amino acids and membrane-spanning domains within their structures.

Table 3
Representative Neural Peptides

Peptide	Subtype	Effector pathways	AA/TMD*
Angiotensin II	AT ₁ , AT ₂	IP ₃ /DAG	359/7, 363/7
Atrial natriuretic	ANP _A , ANP _B	cGMP	1061/1, 1047/1
Bradykinin	B ₁ , B ₂	IP ₃ /DAG	353/7, 364/7
Corticotropin-releasing factor	CRF ₁ , CRF ₂	cAMP	415/7, 411/7
Neuropeptide Y	Y ₁ , Y ₂ , Y ₃ , Y ₄ , Y ₅	cAMP	384, 381, 375, 445, 290/7
Neurotensin	NTS ₁ , nts ₂	IP ₃ /DAG	418/7, 410/7
Opiate receptors	DOP (δ opioid peptide) KOP (κ opioid peptide) MOP (μ opioid peptide)	cAMP	372/7 380/7 400/7
Tachykinins	NK ₁ , NK ₂ , NK ₃	IP ₃ /DAG	407, 398, 468/7
Vasopressin	V _{1a} , V _{1b} V ₂	IP ₃ /DAG cAMP	418/7, 424/7 371/7
Oxytocin	OT	IP ₃ /DAG	389/7
VIP (vasoactive intestinal peptide)	VPAC ₁ , VPAC ₂	cAMP	457/7, 438/7

*Number of amino acids (AA) in the receptor/number of transmembrane-spanning domains (TMD). The atrial natriuretic peptide receptor has a single TMD and contains a guanylate cyclase domain.

4. CONCLUSION

The multitude of neurotransmitter receptors that have been discovered and characterized to date belong to surprisingly few major gene families. However, a given transmitter can produce a surprisingly complex set of responses due to presence of often several subtypes of receptors that activate a variety of effector systems. In several cases, transmitters activate receptors that belong to entirely different families, such as ligand-gated channel and GPCR subtypes. The receptor-effector coupling system offers extensive diversity for biologic actions and remarkable specificity despite its use of a limited number of chemical messages. With the recognition that receptors operate as rheostats rather than as switches and that the number of receptors expressed on the cell surface is dynamically regulated, we have increased our understanding of how receptor-mediated signaling can be fine tuned and have opened the door for new therapeutic approaches.

Acknowledgment: This chapter incorporates material from the chapter “Pre-synaptic Receptors” authored by the late Dr. David K. Sundberg in the first edition of this work.

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6

Neuroembryology and Neurogenesis

G. Jean Harry and Christina T. Teng

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1. INTRODUCTION

It is the intention of this chapter to convey an impression of the overall organizational development of the nervous system. The treatment of the subject will not be exhaustive, and only a few major substructures will be outlined particularly as they may be important or contribute to other chapters in this volume. This chapter first introduces the embryonic development of the neural plate into the neural tube and then discusses the proliferation, differentiation, and migration of the neuronal and neural crest cells. Finally, the concept of apoptosis as a method to refine the circuitry of the brain and the maintenance of adult neurogenesis and injury-induced neurogenesis will be introduced as areas for future consideration.

The nervous system comprises two components: the central nervous system, which is composed of the brain and the spinal cord, and the peripheral nervous system, which is composed of ganglia and the peripheral nerves that lie outside of the brain and the spinal cord. The vertebrate nervous system is considered to have

five major divisions: the telencephalon, diencephalon, mesencephalon, metencephalon, and myelencephalon. These divisions reflect changes that the brain undergoes during its embryologic development.

The development of the nervous system begins in the fetus and, for humans, is not complete until approximately the time of puberty. The complex architecture of the brain requires that different cell types develop in a precise spatial relationship to one another. Organogenesis for the nervous system occurs during the period from implantation through midgestation with synaptogenesis and myelination predominant during late gestation and the early neonatal period. Neurogenesis is a complex process involving proliferation, migration, differentiation, and survival. It is characterized by an expansion phase in which stem cells undergo massive symmetric divisions followed by periods where expanded precursor cells give rise to differentiating cells. This developmental process is highly complex with very specialized morphologic and biochemical patterns that are formed and continue following a critically timed multistage schedule that is guided by chemical messengers. The nervous system framework is established in a sequential process with each step dependent upon the proper completion of

the previous step. During this period of active growth, intricate cellular networks are formed that also follow a specific sequence of events for final cell development and contact with other cells in the nervous system.

2. EMBRYONIC DEVELOPMENT OF THE NERVOUS SYSTEM

2.1. Early Development of the Neural Tube

The nervous system is one of the first structures to appear in the human embryo. *Primary neurulation* refers to the formation of the neural tube and results in the formation of the brain and spinal cord (Table 1). Starting at the third week of development, the *neural plate* is a thickened dorsal *ectodermal plate*. Initially, the lateral edges of the plate elevate to form the *neural folds*, creating a linear groove between them. After constant induction by the adjacent *notochord*, the neural folds become more elevated and fuse with each other in the midline. Differential changes in cell morphology result in the edges of the neural plate folding in to form the neural groove and adhere to each other to form the *neural tube*. The folding of the neural plate involves a coordinated functioning of the cytoskeletal filaments, microtubules, and microfilaments. In addition, this process involves the interaction of various surface glycoproteins, extracellular matrix proteins, and cell adhesion molecules to ensure cell-cell recognition and adhesive interactions between the lips of the neural folds. This tube becomes separated from the rest of the ectoderm (Fig. 1). The fusion of the tube starts in the cervical region but is delayed at the cranial and the caudal ends of the embryo. The first fusion process of the neural tube occurs at approximately 22 days of

gestation forming the lower medulla. This fusion continues with the anterior end of the neural tube closing at approximately 24 days and the posterior end at approximately 26 days at the lumbosacral level of the

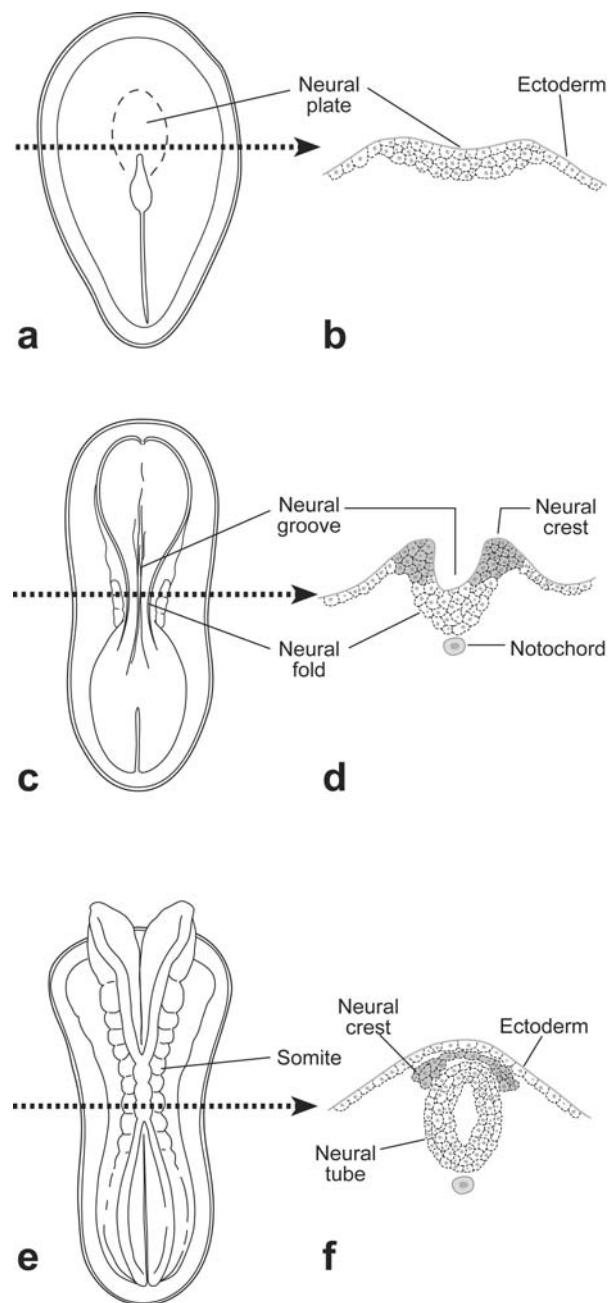


Table 1
Derivatives of Regions of the Neural Tube

Region	Derivative
Dorsal plate	Roofs of the third, fourth ventricles. Tela choroidea (a vascular plexus over the roof of the ventricle).
Dorsolateral plate	This region makes up the bulk of the CNS, deriving association neurons receiving sensory input.
Ventrolateral plate	Motor neurons (neurons whose axons end on skeletal muscle fibers or postganglionic neurons).
Ventral plate	No significant derivatives

Fig. 1. Stages in the formation of neural tube and neural crest: (a, b) the neural plate on the dorsal side of the embryo. (c–f) the formation of the neural groove and neural tube. The neural crest forms an intermediate zone between the neural tube and surface ectoderm. The notochord is located on the midline of the embryo just ventral to the developing neural tube.

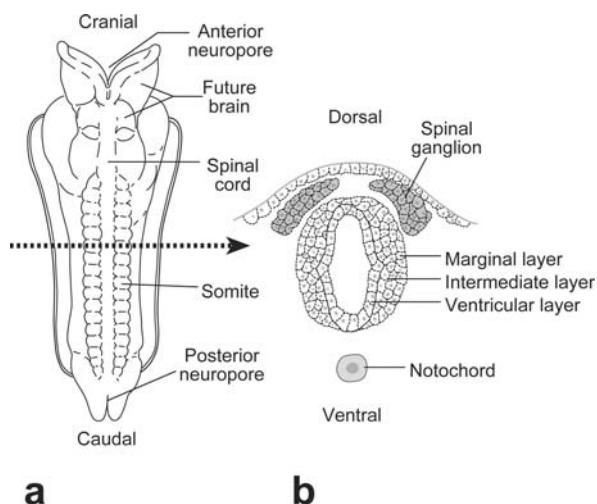


Fig. 2. Formation of neuropores. (a) Dorsal view of a human embryo at 3.5 weeks. A chain of somites is located on each side of the neural tube. (b) Cross section at the *level of the arrow* to show how the neural crest cells develop into spinal ganglion, and the formation of ventricular, intermediate, and marginal layers in the spinal cord.

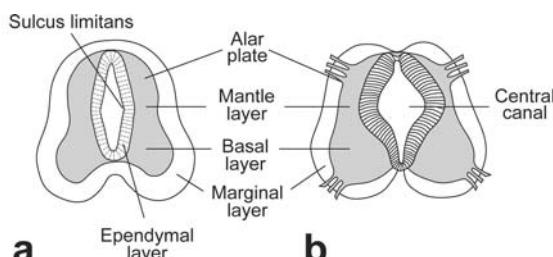


Fig. 3. Development of the human spinal cord. Cross section of the human spinal cord at various stages of development (a) 4-mm (3.5 wk) embryo. (b) 11-mm (5.5 wk) embryo. (c) 30-mm (9 wk) embryo. (d) 80-mm embryo.

spinal cord. The last parts of the neural tube to close are located cranially and caudally and are known as the anterior and posterior *neuropores* (Fig. 2). Once the neural tube has formed, neuroepithelial cells proliferate, and four basic embryonic zones are formed: the ventricular, marginal, intermediate, and subventricular zones. In the human, these are normally formed by the end of the fourth embryonic week. The neural tube forms the CNS, with its hundreds of billions of *neurons*, and can be classified into six major divisions: (1) spinal cord, (2) brain stem, (3) pons and cerebellum, (4) mesencephalon, (5) diencephalon, and (6) cerebral hemispheres.

2.1.1. PROLIFERATION AND DIFFERENTIATION OF THE NEUROEPITHELIAL CELLS

In vertebrate species, the wall of the newly closed neural tube consists of the neuroepithelial cells—a group of multipotent progenitor cells. They form a thick layer of pseudostratified epithelium over the entire wall of the neural tube and divide rapidly, resulting in the production of a neuroepithelial layer. The cells in this layer give rise to the primitive nerve cells or neuroblasts. Neuroblasts represent the first stage in the differentiation of the neurons, migrate away from the lumen, and settle into other portions of the neural tube. They form the mantle layer that eventually becomes the gray matter of the

spinal cord. The neuroblasts pass successively through apolar, bipolar, and unipolar to multipolar stages. The unipolar cell grows out of the mantle zone to become a part of the marginal layer below the external limiting membrane (Fig. 3a). This process becomes the axon of the nerve cell and may synapse with dendrites of other nerve cells within the spinal cord, or it may leave the neural tube as motor fiber growing peripherally to contact muscle cells and glands. In addition to the neuroblast cells, the neuroepithelial cells also give rise to the *glioblasts* and *ependymal cells*—the supportive cells of the nervous system. The *glioblasts* differentiate into both *astrocytes* and *oligodendroglia cells*. Ependymal cells remain in the innermost ventricular layer and form the epithelial lining of the neural canal.

2.2. Formation of Neural Crest and Migration of Cells

During the fusion of the neural plate, a group of neural-plate cells escapes into the space between the tube and the surface of ectoderm (Fig. 1f). The embryonic ectoderm derives the neural crest from which all neurons and glial cells of the nervous system originate.

After the formation of the neural tube, the neural crest appears as two longitudinally running bands of cells on either side of the spinal cord. Cells emerge from the neural crest and migrate away from their original locations to specific sites in the periphery according to characteristic migration pattern determined by the local environment and become widely distributed throughout the body. Cell adhesion and extracellular matrix molecules offer a regulatory effect on neural crest cell motility and morphology. Some neural crest derivatives retain developmental plasticity, and the commitment of terminal differentiation is delayed. These *neural crest* cells give rise to the sensory ganglia of the spinal and cranial nerves and to the postganglionic autonomic neurons (Fig. 2b). Other cells derived from the neural crest cells are the *chromaffin cells* of the adrenal medulla and the *glial cells* in the peripheral nervous system as well as *melanoblasts*. Cells, tissues, and organs that either contain or are constituted of neural crest-derived cells are listed in Table 2. One stream of the cells migrates dorsolaterally into the superficial ectoderm dorsal to the neural tube and the *somites*. These are the precursors of the pigment cells. The other branch of the neural crest cells migrates ventrally between the neural tube and the somites. They will develop into the *sensory ganglia* of the cranial

nerves, the posterior roots of the spinal nerves, as well as the *ganglia* of the *autonomic nervous system*.

Although the actual underlying mechanisms of neuronal migration remain unclear, there are certain factors that have been identified to be important for the successful completion of this process during brain development. (i) The migratory routes are predictable from a given species, yet the pathways of migration can vary from species to species. (ii) Given that a fully established basal lamina overlying the cells is impenetrable to the cells, the presence of an incomplete *basal lamina* is important for the initiation of cell migration. (iii) The components of the *extracellular matrix* (ECM) play important roles in the cell migration. Fibronectin, laminin, and collagen types I and IV promote migration; whereas collagen types II, V, and IX, aggrecan, and versican inhibit migration. Moreover, neural crest cells contribute to the synthesis of some ECM components, given the requirement of sufficient cell-free extracellular space for migration. Neurons also produce proteases and plasminogen activator to create a migration path through the ECM. (iv) Diminished cell-cell gap-junction communication precedes the onset of migration. The loss of *cellular adhesion molecules* (N-CAM, or N-cadherin) was found after initial migration of the cells. However, specific cadherins are expressed in various subpopulations to provide signals for interaction in the migrating neurons. (v) The temporal and spatial expression of growth and cell signaling factors are critical for the initiation, maintenance, and termination of migration. For example, transforming growth factor β (TGF) 1 and 2 and their related protein Dorsalin-1 were found to stimulate neural crest cell migration. A similar effect in this regard has been demonstrated by the transcription factor PAX-3. Any defect in PAX-3 gene (or Splotch mutant) severely delays the onset of neural-crest migration from the neural tube. Other motility-inducing factors, such as hepatocyte growth factor/scatter factor (HGF/SF), are also involved in cell motility.

During the process of migration when the cells run into physical barriers such as basal laminae, blood vessels, and somitic cells in the head or trunk region, they stop migration and begin accumulation. The cessation of migration and the settlement of neural crest cells in their final sites are determined by factors intrinsic to neural crest cells, or by the extracellular microenvironments they encounter. For example, in certain final sites, tissues express versican, which inhibits the function of the molecules (e.g., fibronectin, laminin, and collagen type I) and consequently stops the

Table 2
Cell Types, Tissues, and Organs That Either Contain or Are Constituted by Neural Crest-Derived Cells

Cell types	Tissues or organs
Adipocytes	Adrenal gland
Adrenergic neurons	Blood vessels
Angioblasts	Cardiac septa
Calcitonin-producing (C) cells	Connective tissue of glands (thyroid, parathyroid, thymus, and pituitary)
Cardiac mesenchyme	Cornea
Cholinergic neurons	Craniofacial bone
Chondroblasts	Dentine
Chondrocytes	Dermis
Fibroblasts	Eye
Mesenchymal cells	Endothelia
Odontoblasts	Heart
Satellite cells	Smooth muscles
Schwann cells	Spinal ganglia
Sensory neurons	Striated muscles
Smooth myoblasts	Sympathetic nervous system

migration. Furthermore, the abnormalities involved in the production of laminin and collagen type IV prevent the colonization of the cells. This is also known as the *lethal-spotting (Ls/Ls)* mutant in mice.

2.3. Spinal Cord and Brain Stem

After the neural tube is closed, the *neuroepithelial cells* in the tube begin to give rise to the primitive nerve cells or *neuroblasts*. The proliferation of neuroblasts in the *mantle layer* and each side of the neural tube develops into ventral and dorsal thickenings. These thickenings are known as the *basal plates* and the *alar plates*, respectively. As a general rule, the basal plate begins its differentiation before the alar plate. The boundary between these two is marked by a longitudinal groove, the *sulcus limitans*. This sulcus runs along the length of the neural tube and divides the rapidly expanding side wall of the neural tube (Fig. 3a, b). The sulcus limitans remains visible in the lower part of the *brain stem* in the adult. The neuroblasts in the basal plate form the *ventral and lateral gray columns*, which contain somatic and autonomic motor neurons, respectively. The alar plates form the sensory area (or *dorsal gray column*), containing neuroblasts that become *sensory neurons* (Fig. 3c, d).

Spinal nerves are formed by the juncture of a dorsal sensory root made up of processes from neurons located in the dorsal-root ganglia carrying afferent impulses toward the *spinal cord* and a ventral motor root made up of axons carrying efferent nerve impulses away from the spinal cord (see Chapter 2). Most of the axons in the ventral roots arise from somatic and autonomic motor neurons in the ventral and lateral gray columns of the spinal cord. The axons from the ventral roots will elongate via migration of a growth cone structure at the leading edge and innervate the striated muscle masses that are functionally classified as somatic efferent fibers. Between the dorsal and ventral roots, the lateral column is developed in the thoracic and upper lumbar regions of the spinal cord. The axons from the neurons of the lateral column make up part of the spinal nerves. They grow toward the periphery in the trunk region and synapse with the neural crest-derived neurons in the sympathetic ganglia. The *chain ganglia* of the sympathetic division of the *autonomic nervous system* are connected to the spinal cord by the *gray ramus communicans* and the *white ramus communicans*. The axons of the spinal cord neurons are known as *preganglionic fibers* and those of the ganglia are termed *postganglionic fibers*. The neurons of the parasympathetic division of the autonomic nervous

system are located in the cervical and sacral regions of the spinal cord. The axons from these neurons elongate to synapse with the postganglionic neurons in the wall of the gut.

In the 3-month human embryo, the spinal cord extends the entire length of the embryo. After that time, the *vertebral column* and the *dura* grow more rapidly than does the spinal cord itself, gradually increasing the length of the vertebral column relative to the cord. The terminal end of the spinal cord will eventually shift to a higher level than the corresponding vertebra (Fig. 4a–c). Consequently, the lower end

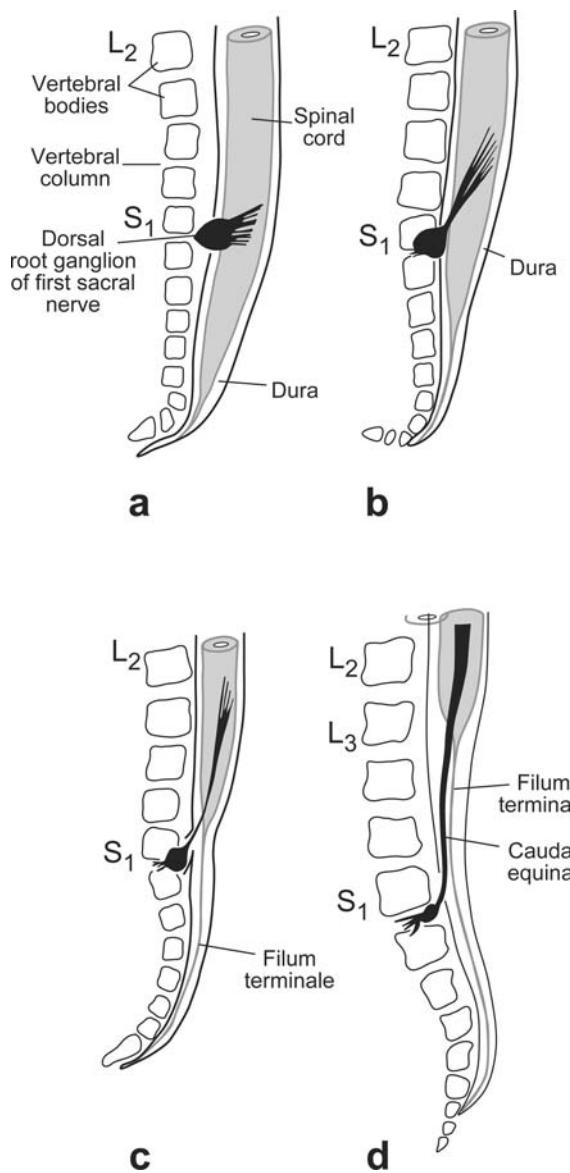


Fig. 4. Formation of the cauda equina. The relationship between the lengths of the spinal cord and the vertebral column at various stages of development. **(a)** third month. **(b)** fourth month. **(c)** fifth month. **(d)** newborn.

of the cord is at the level of the third lumbar vertebra at full term. This differential growth continues after birth, and the spinal cord eventually terminates at the level of the second lumbar (L_2). The dorsal and ventral nerve fibers of the *lumbar* and *sacral* segments that exit from the terminal end of the cord are known as the *cauda equina*. The caudal end of the neural tube remains attached to the *coccygeal* section of the vertebral column, which is a thin filament known as *filum terminale* (Fig. 4d).

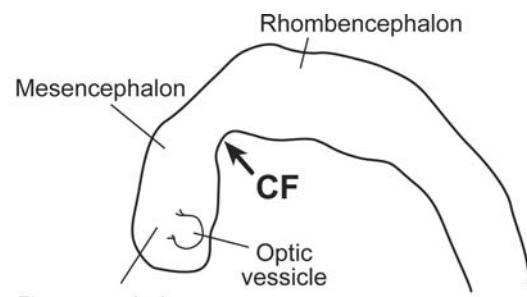
2.4. The Primary Brain Vesicles

After the anterior neuropore closes, this region is known as the *lamina terminalis*. This cranial end of the neural tube expands to form the three main divisions of the developing brain, separated by two constrictions. The continued proliferation of the clonogenic neuroepithelial germinal cells cause the outward bulging of the pallial walls to form the cerebral vesicles. The primary brain vesicles are the *prosencephalon* (forebrain), the *mesencephalon* (midbrain), and the *rhombencephalon* (hindbrain) (Fig. 5a). Because of the continuous growth of the three primary vesicles, the originally straight neural tube shows two external flexures, the *cephalic* and *cervical flexures*, during the third and fourth weeks of development. A third, known as *pontine flexure*, occurs during week 5 in the region of the developing *pons*. These flexures divide the midbrain from the hindbrain and the hindbrain from the spinal cord (Fig. 5b, c). The *optic cup* is from an outgrowth of the forebrain, and the brain stem is derived from the midbrain and part of the hindbrain. The *cerebellum* is a hindbrain derivative (Table 3).

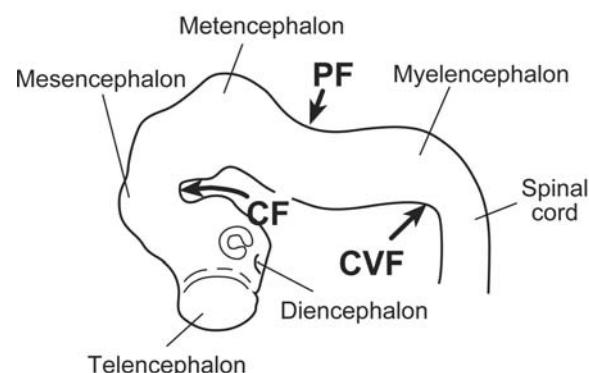
2.5. Myelencephalon and Metencephalon

The development of the *myelencephalon* results mainly from the expansion of the *fourth ventricle* into a large cavity that separates the *alar plates*. With the continued expansion of the fourth ventricle, its dorsolateral plate moves laterally. This stretches and thins the *roof plate*. Consequently, the lateral walls separate the *alar* and *basal* plates by a distinct groove, the *sulcus limitans* (Fig. 6a). Later, three groups of nuclei are derived from each of the *alar* or *basal* plates (Fig. 6b). The *myelencephalon* gives rise to the *medulla oblongata*, which is continuous with the spinal cord (Table 4).

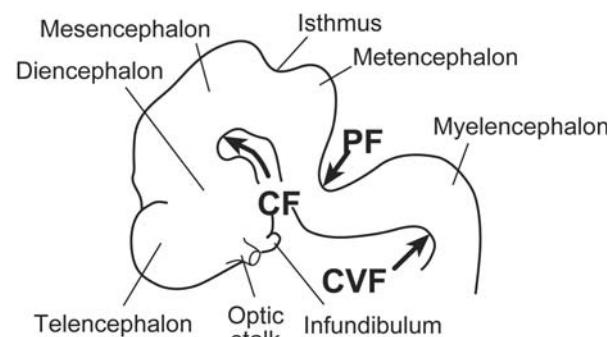
The *metencephalon* consists of a pair of enlarged areas of the dorsolateral plate, the *rhombic lips*, which join dorsally in a midline segment—the



a



b



c

Fig. 5. Changes in the external brain configuration during early development. (a) 3.5-wk embryo. (b) 5-week embryo. (c) 5.5-wk embryo. CF, cephalic flexure; PF, pontine flexure; CVF, cervical flexure.

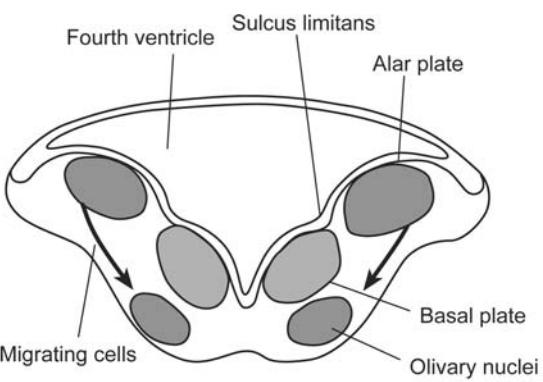
vermis—to form the *cerebellar plate*. The cerebellar plate continues to grow and form the *hemispheres* of the *cerebellum* (Fig. 7). Both the hemispheres and vermis contain a group of intracerebellar nuclei

Table 3
Adult Derivatives of the Early-Brain Vesicles

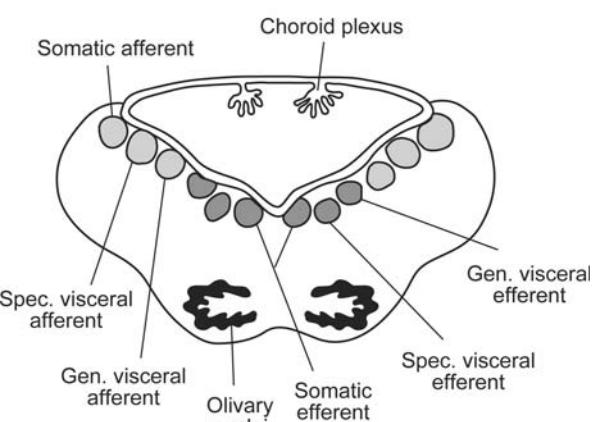
Early brain vesicles (3.5- to 4-week embryo)	Developing brain (4- to 5-week embryo)	Adult derivatives
Prosencephalon (forebrain)	Telencephalon	Cerebral cortex Basal ganglia
	Diencephalon	Epithalamus Thalamus Hypothalamus Subthalamus
Mesencephalon (midbrain)	Mesencephalon	Mesencephalon
Rhombencephalon (hindbrain)	Metencephalon	Pons Cerebellum
	Myelencephalon	Medulla oblongata

and a folded cortex. The ventral portion of the metencephalon will develop into the pons, which consists of a group of *pontine nuclei* originally derived from the alar plate that have migrated to this location (Fig. 7a). The function of the pontine nuclei is to serve as the center for communication by sending their axons to the cerebellum. Blood vessels invade the dorsal plate and form the *choroid plexus*.

Within the cerebellar cortex, a radial migration of cells takes place during early development. During the eighth week, the cerebellar plates constructed by three cellular layers—the *neuroepithelial*, *mantle*, and *marginal* layers—is established. Neuroepithelial cells first migrate to the surface of the cerebellum, where they form the *external granular layer*. The daughter cells of this layer migrate inward to pass the *Purkinje cells* and form the *internal granular layer*. After birth, the cortex of the cerebellum consists of a *molecular layer*, which is occupied by the dendrites of the Purkinje cells and *Golgi* neurons, and an internal granular layer beneath the Purkinje cells (Fig. 7b, c). The generation of *neuronal processes* generally occurs after the completion of cellular migration. However, axon growth may precede cell migration in some cases. For example, in the cerebellar cortex, during the internal migration of granule cells, the cells first extrude in a horizontal process and then leave behind a perpendicular process, giving the axon a T-shaped appearance.



a



b

Fig. 6. Differentiation of alar and basal plates of the myelencephalon. (a) The 5-week embryonic myelencephalon shows the lateral expansion of the fourth ventricle. This results in the formation of alar- and basal-plate nuclei. Arrows indicate the path followed by cells of the alar plate to the olfactory nuclear complex. (b) Three groups of the cranial sensory neurons are derived from the alar plate, whereas cranial motor neurons are differentiated from the basal plate. Olivary nuclei function to make connections with the cerebellum.

2.6. Mesencephalon

In comparison with other developing brain vesicles, the mesencephalon shows no striking structural modification and is soon overshadowed by the cortices of the cerebellum and *cerebrum*. Because of the expansion of the basal and alar plates, the central cavity of the mesencephalon reduces to a narrow channel, the *cerebral aqueduct*, connecting the fourth ventricle of the hindbrain to the *third ventricle* of the diencephalon. The *crus cerebri* (or the base of *cerebral*

Table 4
Afferent and Efferent Components of Cranial Nerves in Conjunction with the Alar and Basal Plates

Origin	Group of nuclei*	Nerves originated
Alar plate	General somatic afferent	V, VII, IX, X
	Special somatic afferent	VIII
	Special visceral afferent	I, VII, IX, X
Basal plate	General visceral afferent	V, VII, IX, X
	General somatic efferent	III, IV, VI, XII
	Special visceral efferent	V, VII
	General visceral efferent	III, VII, IX, X

*In the CNS, a specific clustering of neuronal cell bodies with a functional association is usually called nuclei (a different use of the terminology in comparison with the nuclei of eukaryotic cells).

peduncle), a derivative of the basal plate, connects the descending nerve fibers from the cerebral cortex to the metencephalon and spinal cord. The anterior and the posterior *colliculi* found in the *tectum* of the alar plates serve as the centers for the auditory reflexes and visual impulse (Fig. 8).

2.7. Diencephalon

Early in the development of the diencephalon, the roof plate of the third ventricle is formed by *ependymal cells* and develops into the choroid plexus. The lateral wall of the third ventricle gives rise to the main structures of the diencephalon: the *epithalamus*, *thalamus*, and *hypothalamus*. However, there are no ventrolateral plate derivatives in the diencephalon. In the floor of the third ventricle, three swellings can be identified—the *optic chiasm*, *infundibulum*, and *mammillary body* (Fig. 9a). Later, a pair of *optic vesicles* appears on both sides of the forebrain. They are connected to the ventricle of the diencephalon by the *optic stalk* and become the major portions of the eye.

The epithalamus is the precursor of the *habenular nuclei* and *pineal gland*. A longitudinal groove, the *sulcus hypothalamicus*, appears in the dorsolateral area, separating the thalamus from the hypothalamus. The thalamic areas undergo rapid proliferation and fuse at the midline, and the central cavity is therefore transformed into a slit-like third ventricle. The development and function of the thalamus and *cerebral cortex* are closely interrelated throughout the stages of prenatal and postnatal life. The neuronal nuclei

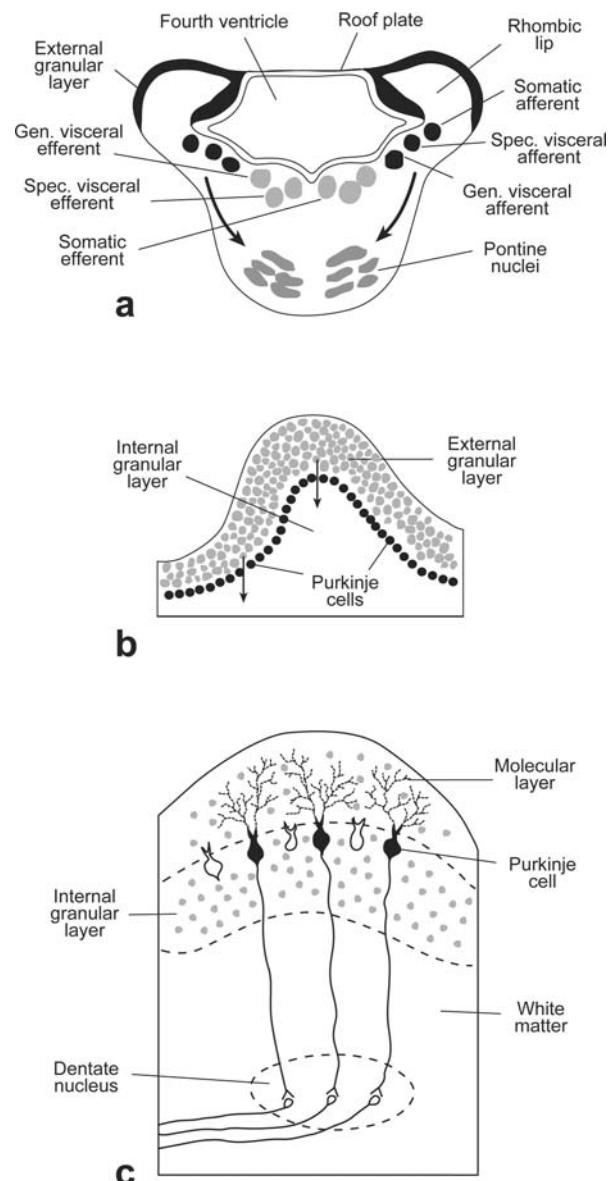


Fig. 7. Development of cerebellum from metencephalon. (a) The rhombic lips expand partly into the lumen of the fourth ventricle and partly into the dorsal direction and give rise to the cerebellum. (b) External granular layer fully formed, and Purkinje cells are positioned in the cortex. The arrows indicate the inward migration of cells to the internal granular layer. (c) Purkinje cells separate the external and internal granular layers. As development proceeds, cells of the external layer migrate inward to the inner layer, leaving an empty layer—the molecular layer.

(groups of neurons) in the hypothalamus are associated with autonomic and endocrine functions. As the neuronal cells continue with proliferation, migration, and differentiation, the three primary brain vesicles develop into the major structures of the adult brain.

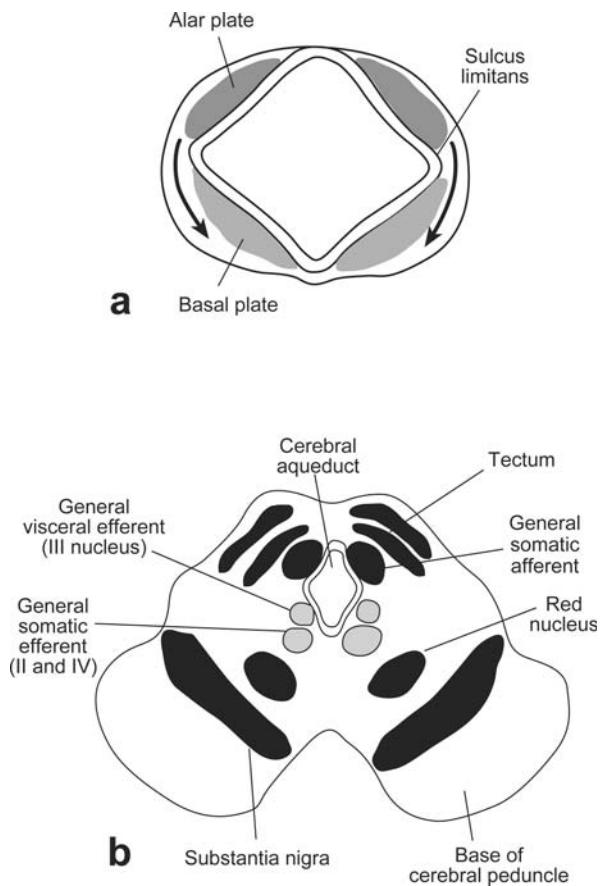


Fig. 8. Differentiation of the mesencephalon. (a) Primitive stage of mesencephalon shows the position of the alar and basal plates. The arrows indicate the path followed by the cells of the alar plate to the red nucleus and substantia nigra. (b) Stage following fusion of ventral part of the cavity and differentiation of nuclei (e.g., tectum and general somatic afferent, general somatic efferent, general visceral efferent nuclei).

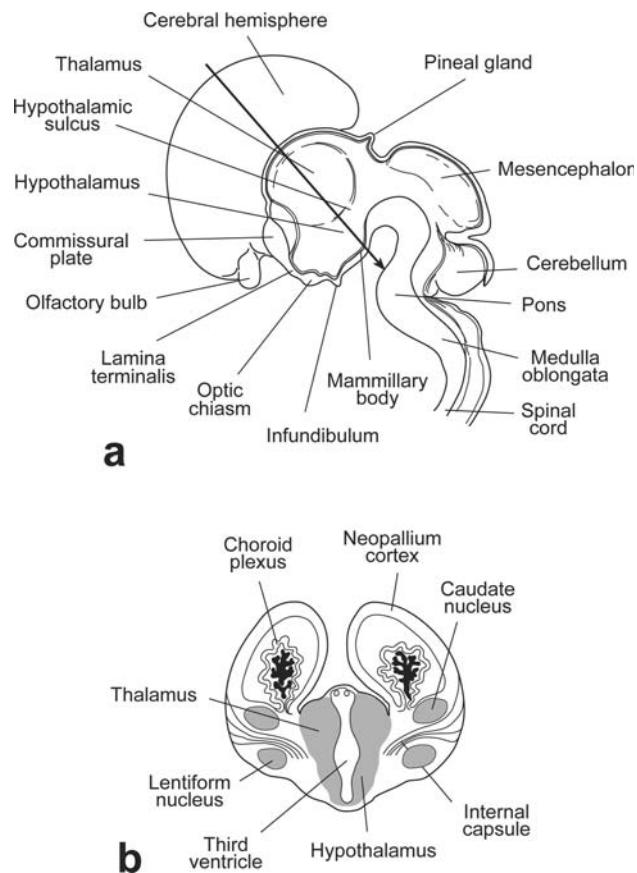


Fig. 9. (a) A sagittal section of the brain in a 10-week-old human embryo. The thalamus and the hypothalamus are derived from alar plates and separated by hypothalamic sulcus. The roof plate of the diencephalon develops into the pineal gland (or epiphysis), whereas infundibulum becomes part of the pituitary gland. (b) Cross section of (a) at the level indicated. The corpus striatum is separated into caudate nucleus and lentiform nucleus by a bundle of internal capsule.

2.8. Telencephalon

The *telencephalon* consists of two cerebral hemispheres and the lamina terminalis. The cavities of the hemispheres form the lateral ventricles, which communicate with the third ventricle. The mantle zone of the basal part of the hemispheres thickens and bulges into the lumen of the lateral ventricles to form the *corpus striatum*. The corpus striatum is later divided into two parts, the *caudate nucleus* and the *lentiform nucleus*, by a bundle of axon fibers (the *internal capsule*) from the cerebral cortex. As development proceeds, the lentiform nucleus changes into the *putamen* and the *globus pallidus*, which are closely associated with the internal capsule together with the caudate nucleus and the thalamus (Fig. 10a). This complex, derived from the corpus striatum, is the center for correlation of sensory impulses and control of motor activity.

Ependymal cells form a thin layer of the hemisphere wall, known as the *pallium*, which becomes the cerebral cortex. Initially, the cerebral cortex exists as two regions: the *neopallium* and the *paleopallium*. In the neopallium, the ependymal cells actively divide into a large number of *neuroblasts* that migrate to the marginal layer and then differentiate into neurons. Consequently, the wall of the neopallium thickens and its neurons become stratified. The histogenesis of the cortical cells follows a distinctive inside-out pattern. That is, the early formed neuroblasts occupy a deep position and form the deeper layers of the cortex, and those formed at later times move to the surface and displace the older cells deeper into the basal layers of the cortex. At birth, a large number of pyramidal cells appear in the motor cortex, and granular cells are found in the sensory area. The choroid

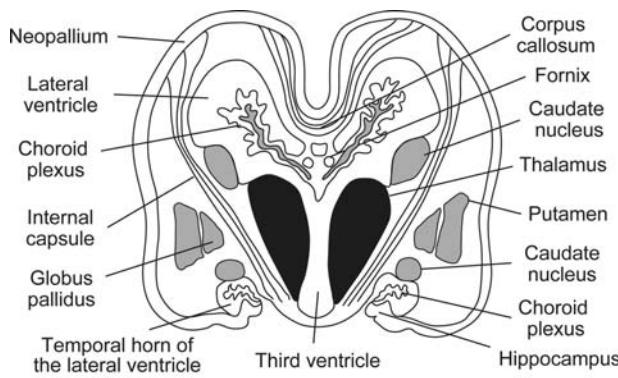
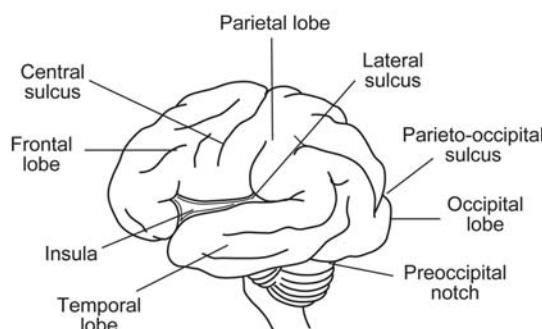
**a****b**

Fig. 10. Development of the telencephalon. **(a)** Cross section of the brain of a 21-week-old human embryo. The lentiform nucleus separates into the putamen and the globus pallidus. The hippocampus alters position due to the growth of the hemispheres. **(b)** Division of the newborn human cerebral hemispheres into five different structural and functional lobes: the *frontal*, *parietal*, *occipital*, and *temporal lobes*, and the *insula*.

plexus and the *choroidal fissure* of the lateral ventricles, are formed from the hemisphere wall attached to the roof of the diencephalon consisting of a single layer of ependymal cells covered by vascular mesenchyme. Just above the choroidal fissure the pallium wall is thickened to form the hippocampus.

As a result of rapid growth of the cerebral hemispheres in anterior, posterior, and dorsal directions in the telencephalon, the formation of numerous folds (*gyri*) and grooves (*sulci*) are created on the surface. The appearance of these folds and grooves serves as the physical landmark to divide the cerebral hemispheres into five different structural and functional lobes: the *frontal*, *parietal*, *occipital*, and *temporal lobes*, and the *insula* (Fig. 10b). Three commissures—the *anterior* and *hippocampal commissure* and the *corpus callosum*—are established in the area of the

lamina terminalis to provide the connection of the two cerebral hemispheres. Among them, the *corpus callosum* is one of the most important and largest commissures. It consists of a large bundle of fibers that crosses the midline of the brain and connects one hemisphere to the other. These fibers extend first anterior and then posterior, thereby covering the dorsal plate of the telencephalon. The plate becomes thicker and serves as the route for the growing callosal fibers. The anterior commissure connects the *olfactory bulb* and the area of the *temporal lobes*. The fibers of the hippocampal commissure arise in the *hippocampus* and form an arching system to connect the mammillary body and the hypothalamus.

3. NEUROGENESIS IN THE EMBRYONIC NERVOUS SYSTEM

3.1. The Origin and Formation of Cortical Neurons

In contrast with neurons in the spinal cord, neurons in the developing cerebellar and cerebral cortices are formed from the migration of postmitotic neuroblasts from the ventricular zones of neuroepithelial cells toward the surface. The cells that arrive in the cortex early remain in the deep layers and become macroneurons. Primitive cells from the germinal matrix proliferate and migrate toward the cortex and then pass over the deep neuronal layers. These cells migrate from the mantle zone through the marginal zone and form a layered sheet of gray matter externally. In the cerebral cortex, two neuronal types are generated in distinct proliferative zones. First, the excitatory pyramidal cells are derived from the neuroepithelial cells in the cortical ventricular zone. Neuroglia originate from the primitive cells of the germinal matrix. They migrate into the intermediate or mantle zone, into fiber tracts, and continue to proliferate. The astrocytes extend their processes and guide the outward migration of neurons from the germinal matrix zones to the cerebral cortex. The radial glial cell processes serve to guide neurons from the zone of neuronal generation to the zones for final settlement, and the laminar features of the cortex are generated over time by differential movement of groups of neurons generated at different times and each neuronal migration sequence bypassing the previous neurons. Thus, the first-generated neurons reach their final position before subsequent generations of neurons. In addition, the radial glial cells also divide asymmetrically to give rise to another radial glial cell while the second cell can differentiate into a

neuron. Once in the cortical plate, neurons are orientated in an inside-out sequence as a result of the migration pattern to form the six-layered structure of the cortex.

The second group of neurons, the inhibitory non-pyramidal cells, are mainly derived from the ganglionic eminence of the ventral telencephalon. Relatively few nonpyramidal cells are derived from the cortical neuroepithelium. These neurons use tangential migratory paths to reach to the cortex. They may follow distinct pathways along axonal bundles of the corticofugal fibers to accumulate in the cortex as a layer of the cortical interneurons. Because the nonpyramidal cells first appear in the marginal zone, some of them form Cajal-Retzius (CR) cells that, through the release of reelin, enhance the migration of pyramidal neurons. A recent discovery indicates that in the mouse embryo, CR cells produce EMX2 protein (a product of Emx2 homeobox gene), which in turn stimulates the synthesis and release of reelin in the CR cells.

The migration of neuronal precursors plays a role in establishing the identity of some neurons and defining the functional properties and connections of the neuron. This translocation is achieved by a combination of the extension of cell process, attachment to the substratum, and subsequent pulling of the entire cell by means of contractile proteins associated with an intracellular network of microfilaments. Directional control occurs as cells move along “guide” cells or according to a concentration gradient of chemotropic molecules. The subplate neurons elaborate a dendritic arbor with spines, receive synaptic inputs from ascending afferents from thalamus and distant cortical sites, and extend axonal collaterals to overlying cerebral cortex and to other cortical and subcortical sites. The subplate neuron layer in frontal human cortex reaches a peak number between approximately 23 and 24 weeks of gestation. Programmed cell death of this layer appears to begin late in the third trimester with a loss of the majority of the subplate neurons by 6 months of age. For both the somatosensory and visual cortices, a similar pattern is followed slightly later in life. It is these processes that coordinate the formation of the final neural network.

3.2. Extracellular Molecules for Developing Neurons

As extracellular signaling molecules, growth factors have diverse effects on neurogenesis, proliferation, and maintenance of new neurons. Cell surface adhesion and recognition molecules mediate interactions between individual cells and between cells and

the extracellular matrix. Additional interactions occur by means of diffusible molecules such as growth factors and trophic agents. The growth and survival of neurons are determined by the presence of a family of intrinsic factors. These are nerve-growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin (NT-3, NT-4/5, and NT-6), and epidermal growth-factor (EGF)-receptor family (ErbB, ErbB2, ErbB3, and ErbB4). These factors mediate their action on responsive neurons by binding to the cell-surface receptors. For example, all neurotrophins bind to the neurotrophin receptor (p75), and NGF, BDNF, and NT-3 bind to TrKA, TrKB, and TrkC (receptor tyrosine kinases), respectively. Nerve growth factor (NGF) is required for survival and neurite outgrowth of cholinergic neurons of the basal forebrain, sympathetic postganglionic neurons, and sensory ganglion cells derived from the neural crest. BDNF contributes to brain synaptic plasticity and promotes neurogenesis and cell survival and has its maximum effect during the time when embryonic neurons contact targets in the CNS. This growth factor assists in maintaining the basal activity in the proliferative zones of the brain and can directly stimulate neurogenesis. Extrinsic factors also exist outside the CNS and include several fibroblast growth factors (FGFs), leukemia inhibitory factor (LIF), insulin-like growth factor (IGF), and platelet-derived growth factor (PDGF). EGF is known to be important in the proliferation and maintenance of embryonic and adult neural stem cells, and EGFR is involved in the radial migration and maturation of neural precursors during embryonic cortical development. Basic fibroblast growth factor (FGF) is produced by neurons and stimulates outgrowth of neurites. IGF-1 mediates BDNF action. The effects of these factors are not restricted to specific neuronal populations, and each type of neuron is influenced by several growth factors. In general, neurons change their trophic factor requirements throughout development; for instance, the bone morphogenetic proteins (BMPs), which enhance the growth of astrocytes yet suppress the growth of oligodendroglia.

Proneural basic-helix-loop-helix (bHLH) transcription factors drive neurogenesis via cell cycle exit-specific protein expression. The Sox B1 subfamily of the HMG-box transcription factors (Sox 1-3) is expressed by precursors in the embryonic nervous system. They are expressed by most progenitor cells of the developing CNS and are downregulated when the cells exit the cell-cycle and differentiate. Some bHLH genes are involved in neural determination,

and others such as Mash1 and NeuroD are involved in terminal neuronal differentiation. The proneural protein, Mash1 (mammalian achaete-scute homolog), is essential to the production of neurons in the embryonic ventral telencephalon. As with other neurogenin family proteins, Mash1 promotes commitment of multipotent progenitors to neurons and inhibits astrocyte differentiation.

Migration is influenced by the adhesion properties of the cells and the direct interactions between a cell and the extracellular matrix. These include cell adhesion molecules (CAMs), intercellular adhesion molecules (I-CAMs), integrins, and cadherins. CAMs are a family of high-molecular-weight cell-surface glycoproteins with regulatory properties during neural development. Family members include neural CAM (N-CAM), neural-cadherin (N-CAM), neuronal-glia CAM (Ng-CAM-NILE or L1), tenascin, and adhesion molecule on glia (AMOG/beta2 isoform of the membrane Na, K-ATPase pump). N-CAM is widespread early in embryogenesis and in both neurons and glia throughout nervous system development. As such, it may contribute to general processes involving glial guidance of axonal processes, neurite fasciculation, axon-target cell interactions, and cell positioning relationships. N-cadherin is expressed early in the ectoderm and may serve to induce the development of the neural plate and mediate closure of the neural tube. It is also expressed later in development providing guidance cues for growth cones during neurite extension. Integrins are membrane receptors with ligands consisting of I-CAMs and other matrix components such as collagen, laminin, and fibronectin. Integrin activation can lead to rapid changes in cell adhesion properties in the local environment and can signal intercellular events. These receptors provide the developing neural cells a system for linking adhesion/migration information with other developmental signals controlling proliferation and differentiation.

4. NEURONAL APOPTOSIS IN THE DEVELOPING NERVOUS SYSTEM

4.1. The Function of Neuronal Apoptosis

Refinement of the final neural network requires that redundant or inappropriate cells and their connections be terminated; thus, requiring a period of apoptosis and neural pruning. During the embryonic proliferation phase, neurons are generated in excess to ensure the appropriate number of neurons for proper innervation of target sites. This neuronal

death is a genetic *programmed cell death* or *apoptosis*. Apoptosis is one of the major events in the process of *neurogenesis* (e.g., cellular proliferation, migration, differentiation, and cell death) and can occur in proliferating neural precursor cells, recent postmitotic cells, or in differentiated neurons during the formation of the nervous system (Table 5). The early form of cell death may eliminate unwanted precursor cells with inappropriate phenotypes or may serve a critical role in creating the environment for pattern formation in the nervous system. Neuronal apoptosis is not uniform throughout the nervous system. A higher rate of apoptotic neuronal death was found in the primordial cortex (90%) than in the spinal cord, where approximately 50% of the motoneurons die. A similar rate of cell death was found in the interneurons of the retina.

Developmentally related apoptosis has been demonstrated to (i) eliminate the neurons that have made inappropriate synaptic connections with other neurons or their own targets (this is to ensure optimal numerical relationships between neurons and target cells); (ii) remove the neurons that serve transient developmental functions or those located in the *ectopic sites*; (iii) facilitate the *pattern formation* and *morphogenesis* of the CNS in early development; and finally, (iv) serve as a natural innate control mechanism within the neuron that is able to react to the changes in its surrounding environment such as shortage of nutrient, a lack of trophic factors, and an altered endogenous endocrine state. Thus, the apoptotic death of neurons has frequently been interpreted as their failure to compete for limited amounts of population-specific target-derived trophic factors (or the *neurotrophic theory*).

Table 5
Localization of Early Neural Apoptosis

<i>Developmental stage</i>	<i>Location</i>
Neurulation	Neural plate, neural fold, and neural tube
Neural crest formation	Premigratory and migratory neural crest cells
Eye induction and formation	Forebrain, optic vesicle, and optic cup
Early neurogenesis	Neural tube and spinal cord
Mid neurogenesis	CNS, cerebral cortex, retina, and peripheral nervous system (PNS) ganglia

4.2. Underlying Mechanisms of Regulating Developing Neuronal Apoptosis

Apoptosis is an important process of neurogenesis in the developing CNS, thus the regulation of factors to promote apoptosis as well as to offer protection from apoptotic signaling is critical. In the embryonic stage, the intrinsic neurotrophins are the major factors that regulate neuronal survival during the period of active programmed cell death. The source for such factors often is related to neuronal interactions with non-neuronal cells such as glia via cell contact, secreted factors, and gap-junction communication. For instance, the withdrawal of NGF from cultured sympathetic neurons shows the characteristic cell death pattern in the dying neurons (e.g., chromatin condensation, cell shrinkage, membrane blebbing, the elevation of caspase-3 activity, a laddering type of DNA fragmentation, and *de novo* protein synthesis). The removal of NGF results in a decrease in the basal activity of the mitogen-activated protein kinase (MAPK), followed by a series of metabolic changes, including the increased production of reactive oxygen species (ROS), decreased glucose uptake, and decreased RNA and protein synthesis.

During the initiation phase of neuronal apoptosis, the c-Jun N-terminal kinase (JNK) and *p38* MAPK are activated. These kinases consequently induce a sustained activation of c-Jun proteins. Consistently, high levels of c-Jun are generally required to serve as a regulator for the *de novo* protein synthesis that is needed for apoptotic cell death. In the late apoptotic phase, some biochemical events converge on mitochondria as a common pathway. These events include the loss of mitochondrial membrane potential, generation of ROS, calcium flux, and the release of cytochrome c for caspase activation. Recently, 14 caspases have been identified as the aspartate-specific cysteine proteases, of which caspase-3 and -9 appear to play significant roles in the developing CNS. Caspase-3, a member of the executioner caspases, can proteolytically cleave a number of cellular proteins, resulting in the morphologic feature of apoptosis.

In addition to the various neurotrophic factors available in the brain from either the neuron directly

or from the surrounding glia, steroid hormones such as sex steroids and adrenal cortical hormones have been shown to offer a level of protection to neurons. As in other cells, the final outcome is a balance between the various antiapoptotic and proapoptotic genes' products of the *bcl-2* gene family, which determine neuronal susceptibility to apoptosis. During CNS development, Bcl-XL protein appears to be the antiapoptotic member, whereas Bax protein emerges as the proapoptotic member of the Bcl-2 family. They are both capable of forming heterodimers and therefore through their interaction may determine neuron survival or death.

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Disorders of Neuronal Migration

Gregory Cooper, Gerald Eichhorn and Robert Rodnitzky

Abnormalities in the process of neuronal migration during embryogenesis result in CNS structures that are dysfunctional, architecturally abnormal, or totally absent. Several distinct mechanisms can be operative in the pathogenesis of clinical disorders related to disrupted neuronal migration. Some neuronal migrational disorders are heritable, and others are presumed to be the result of ischemic, toxic, or metabolic damage during the perinatal period.

CORTICAL NEURONS IN NEURONAL MIGRATION DISORDERS

The most common neuronal migration abnormalities involve the neocortex. In humans, normal neuronal migration to the cerebral cortex takes place mostly during the 8th to the 24th week of gestation. Neurons originating deep in the brain along the surface of the ventricles migrate to the cortex along a network of extensions of glial cells known as radial glia. The first neurons to arrive populate the deepest layer of the cortex, and successive groups of arriving cells occupy progressively more superficial positions. It has been postulated that some structural abnormalities of the cortex are the result of perinatal insults that have caused the death of radial glia or disruption of the network they normally form. The end result is a group of conditions characterized by abnormal laminar or columnar neuronal cortical architecture. In these conditions, a variety of abnormalities are often apparent on gross inspection of the brain, including decreased overall brain size and weight (e.g., microcephaly), abnormally small cortical gyri (e.g., polymicrogyria), or, more commonly, enlarged gyri (e.g., macrogryria). Microscopically, abnormal clusters of misplaced neurons (e.g., heterotopias) can also be seen. When the overall size and weight of the brain

is subnormal, it suggests that there may have been an insufficient number of cells migrating rather than merely misguided migration. Abnormal formation of the corpus callosum is commonly associated with disordered neuronal migration and can be readily appreciated on gross inspection of the cut brain.

Many of these gyral and callosal abnormalities can be identified in life through the use of magnetic resonance imaging (MRI). This enhanced ability to identify such abnormalities has proved that they are much more common than once believed. Before modern neuroimaging, only patients with severe neurologic dysfunction (e.g., epilepsy, mental retardation, weakness, and incoordination) were identified as being afflicted with a neuronal migration disorder, usually at autopsy. It is now understood that similar, but much milder, clinical syndromes can occur and affect a far greater number of persons.

EPILEPSY AS A SYMPTOM OF ABNORMAL CORTICAL NEURONAL MIGRATION

Neuronal migration abnormalities can be so severe that they are incompatible with life or so mild that they are asymptomatic. Among those who survive with these disorders, epilepsy is the most common neurologic symptom. Even small foci of abnormally placed cortical neurons can sufficiently disrupt normal interneuronal physiology to result in epilepsy. The convulsive seizures associated with neuronal migration disorders are often relatively refractory to medical therapy. The modern assessment of a newborn infant who demonstrates a failure to thrive and intractable epilepsy includes a neuroimaging evaluation to investigate the possible presence of one of the cortical dysplasias related to abnormal neuronal migration.

KALLMANN'S SYNDROME AS A PROTOTYPE OF THE HERITABLE DISORDERS OF NEURONAL MIGRATION

It is estimated that between 5% and 20% of neuronal migration abnormalities are genetic in origin. Kallmann's syndrome is a heterogeneous developmental genetic disorder affecting about 1 in 8000 males and 1 in 40,000 females, with the majority of cases being sporadic.

It is characterized by an inability to smell and underdevelopment of gonadal function. The impaired olfaction is related to lack of development of the olfactory bulbs and tract, and the hypogonadism is caused by deficiency of a hypothalamic hormone, leuteinizing hormone-releasing hormone (LHRH), which is critical to the development of the male gonads. The neurons that ultimately secrete LHRH are formed in the olfactory placode and then migrate from the placode up the nervous terminalis into the forebrain behind the olfactory bulb, and ultimately up the olfactory tract into the hypothalamus. The abnormalities are caused by defective neuronal migration of olfactory neurons and of neurons producing gonadotropin-releasing hormone. Both classes of neurons probably share a common origin in the migration pathway, which explains the linkage of these seemingly disparate anomalies.

The isolation of an X-linked Kallmann's syndrome gene, KAL1, may shed considerable light on the

pathogenesis of inherited disorders of neuronal migration. The protein of this gene shares homology with neural cell adhesion molecule, which suggests that the gene may influence neuronal migration. This notion has been supported by the discovery that this gene was deleted in a patient with Kallmann's syndrome.

Mutations of another gene called filamin 1 (FLN1) on the X-chromosome can lead to subependymal heterotopias. The product of this gene is an actin filament cross-linking protein that also links membrane proteins to actin. It is speculated that filamin provides a link between membrane receptors and the actin cytoskeleton of the neuron, allowing proper migration along the radial glia from the germinal matrix to the cortex.

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1. INTRODUCTION

The human brain is a highly metabolic organ with no effective mechanism for storage of oxygen and glucose. The brain needs a constant supply of large amounts of blood, and blood flow is autoregulated by the brain itself. The brain comprises only 2% of total body weight but uses 15% of cardiac output and 25% of total oxygen consumption. The vasculature of the brain and spinal cord consists of an arterial input, intervening capillaries, and a venous drainage system. There are no lymphatic vessels in the central nervous system (CNS).

The intracranial vasculature differs from that found in the rest of the body in several ways. Arteries and veins of the brain pierce the dura mater and arachnoid membranes and lie in the subarachnoid space, bathed by cerebrospinal fluid (CSF).

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All intracranial veins drain into the dural venous sinuses, which do not exist outside the skull. There are also differences in structure, innervation, and functional responses to injury. For example, subarachnoid hemorrhage may have profound and long-lasting effects on the cerebral arteries. The presence of blood in the CSF may induce sustained vasoconstriction in neighboring arteries, whereas blood applied to the tunica adventitia of extracranial arteries does not induce sustained vasospasm. Many fine details about these differences are incompletely characterized, partly because most observations on the nature of neurogenic responses in intracranial vessels have been in animals, and vascular responses to pharmacologic and electrical stimuli vary widely among species.

Intracranial blood vessels are involved in two common diseases of the brain: ischemic lesions (infarcts), which are sites of brain-tissue destruction caused by insufficient or lack of blood supply; and hemorrhages, which are sites of spontaneous rupture of



Fig. 1. Contrast (gadolinium) enhanced magnetic resonance angiography of the aortic arch and brachiocephalic arteries: asterisk, aortic arch; 1, Innominate Artery; 2, Left Common Carotid Artery; 3, Left Subclavian Artery; 4, Right Common Carotid Artery; 5, Right Vertebral Artery; 6, Left Vertebral Artery; 7, Left Internal Carotid Artery; 8, Left External Carotid Artery; 9, Right Internal Carotid Artery; 10, Right External Carotid Artery; 11, Right Subclavian Artery.

intracranial vessels resulting in subarachnoid, parenchymal, or intraventricular extravasation of blood. Epidural hemorrhage is caused by rupture of intracranial meningeal vessels, and subdural hemorrhage is caused by rupture of cortical veins bridging the surface of the cerebral hemispheres and the dural venous sinuses.

1.1. Human Vasculature Can Be Visualized *In Vivo* by Four Different Modalities

The intracranial vasculature is visualized *in vivo* by different techniques, including conventional digital subtraction catheter angiography (Fig. 1), screen film catheter angiography, magnetic resonance angiography (MRA) (Fig. 2; *see also* Fig. 10), computed tomography angiography (CTA), and Doppler ultrasound. The older screen film (Fig. 3) and current digital subtraction techniques are the gold standard for visualizing the intracranial vasculature. These are invasive procedures that involve selectively inserting a catheter into the carotid or vertebral arteries and injecting an iodinated contrast agent. The risk of complications from the invasiveness of the procedure, such as stroke, is less than 1%. Both MRA and Doppler sonography are noninvasive methods for visualizing the intracranial vasculature, which do not involve the injection of contrast medium and use flow within the vessels



Fig. 2. Lateral projection from a selective left common carotid angiogram: 1, Common Carotid Artery; 2, Internal Carotid Artery; 3, External Carotid Artery; 4, Ophthalmic Artery; 5, Posterior Communicating Artery; 6, Posterior Cerebral Artery; 7, Anterior Cerebral Artery; 8, Cortical Branches of Middle Cerebral Artery; 9, Cortical Branches of Anterior Cerebral Artery.

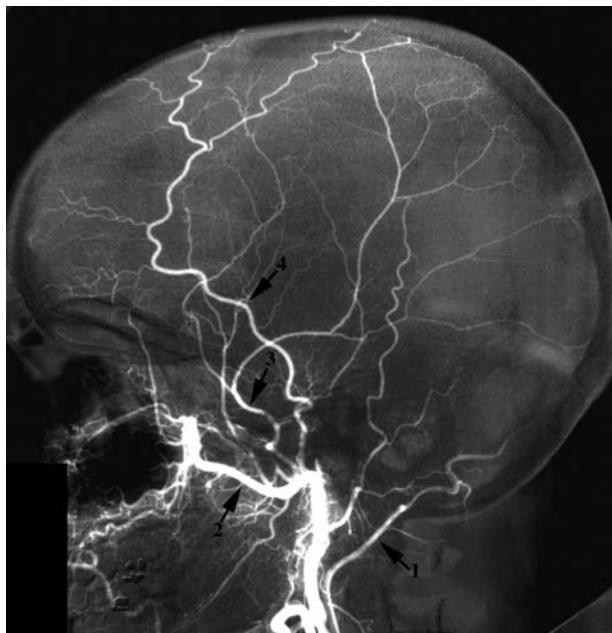


Fig. 3. Selective left external carotid angiogram, lateral projection: 1, Occipital Branch; 2, Internal Maxillary Branch; 3, Middle Meningeal Branch; 4, Superficial Temporal Artery.

to visualize the vasculature. Current refinements in time-of-flight, phase-contrast, and contrast-enhanced methods have made MRA the noninvasive method of choice for evaluating larger intracranial and extracranial arteries and the larger dural venous sinuses. With the advent of faster multidetector computed tomography (CT) scanners, CTA has also proved to be an excellent modality for evaluation of the intracranial and extracranial vasculature. This modality does involve intravenous injection of an iodinated contrast agent but does not require arterial catheter insertion.

The undulating surface of the brain formed by gyri and sulci is responsible for the wavy appearance of the superficial cortical arteries and veins on cerebral angiography.

2. INTRACRANIAL ARTERIAL SYSTEM (TABLE 1)

The intracranial cavity is divided into the supratentorial and infratentorial compartments by the tentorium cerebelli. The contents of the supratentorial compartment include the two cerebral hemispheres, which are supplied mostly by the anterior (carotid) circulation. The contents of the infratentorial compartment, or posterior cranial fossa, include the brain stem and cerebellum, which are supplied by the posterior (or vertebrobasilar) circulation.

Table 1
The Intracranial Arterial System

Intracranial cavity

Tentorium cerebelli divides the intracranial cavity into supratentorial and infratentorial compartments

Falx cerebri subdivides the supratentorial cavity into right and left compartments

Anterior (carotid) circulation

Supratentorial intracranial cavity

Right and left cerebral hemispheres within the supratentorial intracranial cavity are supplied by the internal carotid arteries

Posterior (vertebrobasilar) circulation

Brain stem and cerebellum within the infratentorial intracranial cavity are supplied by the vertebrobasilar system

Posterior cerebral arteries are branches of the basilar artery (posterior circulation) and supply a portion of the cerebral hemispheres (supratentorial compartment)

The great vessels, branches of the aortic arch, supply blood to the head and neck (Fig. 1). The three major branches are brachiocephalic trunk (innominate artery), left common carotid artery, and left subclavian artery (Fig. 1). The brachiocephalic trunk divides into the right subclavian artery and right common carotid artery. The right and left vertebral arteries are branches of the respective subclavian arteries (Fig. 1). Both common carotid arteries bifurcate in the midcervical region, giving rise to the external and internal carotid arteries (Fig. 2). The two internal carotid and two vertebral arteries supply blood to the brain.

The external carotid arteries supply the cervical and facial soft tissues, the sinonasal cavity, the external ear, and the soft tissues of the scalp (Fig. 3). The middle meningeal branch of the external carotid artery enters the intracranial cavity through the foramen spinosum, supplying the meninges.

Intracranial arteries are of two types: extradural or epidural arteries and intradural arteries. The extradural or epidural arteries are also known as meningeal arteries, which are branches of the external carotid artery and supply blood to the meninges and the extradural segments of the intracranial nerves. The intradural arteries are branches of the internal carotid and vertebral arteries and are distributed throughout the intracranial subarachnoid space once those vessels have penetrated the dura mater and arachnoid membrane.

2.1. Anterior (Carotid) Circulation

2.1.1. THE INTERNAL CAROTID ARTERY (TABLE 2)

The internal carotid artery within the neck (cervical segment) has no angiographically visible branches. It enters the skull base via its own canal (carotid canal), located in the petrous portion of the temporal bone (Fig. 2). It then traverses the petrous bone and enters the intracranial cavity, emerging within the cavernous sinus (cavernous segment). The intracranial internal carotid artery is subdivided into the cavernous segment, clinoid segment (at the level of the anterior clinoid process), ophthalmic segment (at the origin of the ophthalmic artery), and communicating segment (at the origins of the posterior communicating and anterior choroidal arteries). The ophthalmic artery is the largest branch of the internal carotid artery (Fig. 2, Fig. 4, and Fig. 5), supplying the eye, orbit, and adjacent paranasal sinuses.

The posterior communicating artery (Fig. 2 and Fig. 5) is an important anastomotic channel for the circle of Willis connecting the anterior (carotid) circulation to the posterior (vertebrobasilar) circulation and connects the internal carotid artery to the ipsilateral posterior cerebral artery. The perforating branches of the posterior communicating artery supply the thalamus, optic tract, and internal capsule. The anterior choroidal artery (Fig. 5 and Fig. 6) is a small, constant branch of the distal internal carotid artery and supplies blood to the optic tract, posterior limb of the internal

Table 2
The Intracranial Internal Carotid Artery

Branches

Meningohypophyseal artery

Inferolateral artery

Capsular arteries

Ophthalmic artery

Superior hypophyseal arteries

Posterior communicating artery

Anterior choroidal artery

Middle cerebral artery

Anterior cerebral artery

Brain structures supplied

Cerebral hemispheres

The eye, optic nerve, optic chiasm, and optic tracts

Midbrain through anterior choroidal artery

Thalamus and internal capsule through posterior communicating artery

Pituitary gland and hypothalamus

Meninges of the skull base

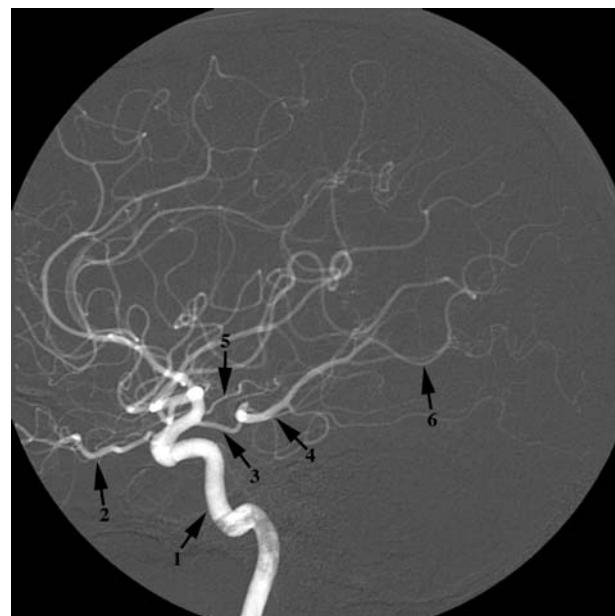


Fig. 4. Selective left internal carotid angiogram, lateral projection: 1, Internal Carotid Artery; 2, Ophthalmic Artery; 3, Posterior Communicating Artery; 4, Posterior Cerebral Artery is a direct continuation of Posterior Communicating Artery (fetal origin); 5, Anterior Choroidal Artery; 6, Cortical Branches of the Posterior Cerebral Artery.



Fig. 5. Selective left internal carotid angiogram, lateral projection: 1, Internal Carotid Artery; 2, Ophthalmic Artery; 5, Anterior Choroidal Artery; 7, Anterior Cerebral Artery; 8, Cortical Branches of Anterior Cerebral Artery; 9, Cortical Branches of Middle Cerebral Artery.

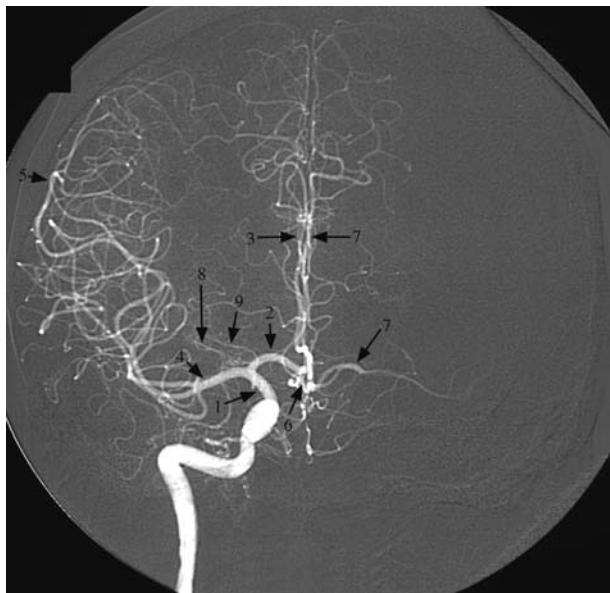


Fig. 6. Selective right internal carotid angiogram, frontal projection: 1, Intracranial Segment of the Internal Carotid Artery; 2, Proximal Anterior Cerebral Artery, A1 Segment; 3, Distal Right Anterior Cerebral Artery; 4, Proximal Middle Cerebral Artery, M1 Segment; 5, Distal Middle Cerebral Artery; 6, Anterior Communicating Artery; 7, Left Anterior Cerebral Artery visualized through Patent Anterior Communicating Artery on Right Carotid Angiogram; 8, Lenticulostriate Arteries; 9, Anterior Choroidal Artery.

capsule, ipsilateral cerebral peduncles, choroid plexus of the ipsilateral lateral ventricle, medial temporal lobe, thalamus, and part of the corpus striatum. The relatively small branches of the intracranial internal carotid artery include the meningohypophyseal artery, inferolateral trunk, and capsular and superior hypophyseal arteries. These numerous small branches supply the meninges of the skull base, the cranial nerves in the cavernous sinus, and the pituitary gland.

The internal carotid artery terminates into the anterior cerebral and middle cerebral arteries (Fig. 6).

2.1.2. THE ANTERIOR CEREBRAL ARTERY (TABLE 3)

The anterior cerebral artery (ACA) is divided into the proximal A1 horizontal segment and distal A2, A3, and A4 segments (Fig. 4 and Fig. 6).

The A1 segment of the ACA extends from the carotid terminus to the level of the anterior communicating artery. The A2 segment of the ACA extends from the anterior communicating artery to the level of the genu of corpus callosum. The A3 segment of the ACA lies over the body of the corpus callosum. The A4 segment of the ACA refers to branches of the ACA lying over the surface of the cerebral hemispheres. The

Table 3
Anterior Cerebral Artery

Branches

Superficial cortical branches

Orbital frontal

Frontal

Parietal

Callosal branches

Pericallosal

Callosal marginal

Perforating branches

Recurrent artery of Heubner

Medial striate or medial lenticulostriate

Blood supply

Medial surface of cerebral hemispheres

Medial and inferior surface of the frontal lobe and medial parietal lobe

Corpus callosum

Caudate nucleus, anteromedial and inferior basal ganglia, internal capsule

Anterior communicating artery

Connects the two anterior cerebral arteries

branches of the proximal A1 segment of the ACA include the small medial striate arteries and recurrent artery of Heubner. The perforating striate branches of the ACA supply the head of the caudate nucleus, anteromedial and inferior basal ganglia, inferomedial internal capsule, and anterior commissure. The cortical branches of the A2 segment of the ACA include two large orbital and frontal cortical branches and supply the inferior and medial portion of the frontal lobe, gyrus rectus, and olfactory bulb and tract (Fig. 4 and Fig. 6).

The branches of the A3 and A4 segments of the ACA include the callosal perforating branches, pericallosal artery, calloso-marginal artery, parietal branches, and terminal cortical branches. The branches of the distal segment of the ACA supply the anterior two-thirds of the medial cerebral hemisphere, the small strip of cortex over the cerebral convexity, and the corpus callosum.

The anterior communicating artery (Fig. 6) connects the proximal segments of both the anterior cerebral arteries in the midline and is an important anterior anastomotic channel within the circle of Willis connecting right and left anterior (carotid) circulations.

2.1.3. THE MIDDLE CEREBRAL ARTERY (TABLE 4)

The middle cerebral artery (MCA) is the larger of the two terminal branches of the internal carotid

Table 4
The Middle Cerebral Artery

<i>Branches</i>
<i>Superficial cortical branches</i>
Anterior temporal
Orbitofrontal
Prefrontal
Precentral sulcus
Central sulcus
Postcentral sulcus
Parietal, angular, temporal, and occipital
<i>Perforating branches</i>
Lenticulostriate arteries
<i>Blood supply</i>
Basal ganglia
Internal capsule
Anterior temporal lobe and most of the lateral surface of the cerebral hemisphere

artery (Fig. 2, Fig. 4, and Fig. 6), and its vascular territory is most commonly involved in thromboembolic ischemic disease of the brain. The proximal M1 segment of the MCA extends laterally to the level of the sylvian fissure and divides into numerous cortical distal branches. The proximal segment of the MCA gives off numerous perforating branches known as lenticulostriate arteries (Fig. 6) and an anterior cortical temporal branch. The lenticulostriate arteries supply most of the caudate nucleus, the basal ganglia, and the internal capsule. The anterior temporal arteries supply the anterior pole of the temporal lobe.

The M2 segment of the MCA runs in the insular or sylvian fossa superiorly, and then arches (M3 segment) underneath the frontal operculum over the temporal lobe horizontally and emerges from the sylvian fissure as the M4 segment and supplies the lateral surface of the cerebral hemisphere. All the branches of the distal segment of the MCA are cortical branches. The orbitofrontal and prefrontal cortical branches supply the inferior surface or the orbital surface of the frontal lobe laterally and the frontal pole of the anterior frontal lobe. The central cortical branches are precentral sulcal, central (rolandic), and postcentral sulcal branches that supply the rolandic area, both anterior and posterior to the central sulcus over the lateral surface of the cerebral hemisphere. The posterior cortical branches of the MCA are the posterior parietal, angular, temporal, and occipital branches. All the cortical branches of the MCA supply the cortical lateral surface of the frontal, parietal, occipital, and temporal lobes (Fig. 2, Fig. 4, and Fig. 6).

2.1.4. THE POSTERIOR CEREBRAL ARTERY (TABLE 5)

The posterior cerebral arteries are the two terminal branches of the basilar artery (Fig. 7 and Fig. 8). Although the posterior cerebral artery (PCA) is part of the vertebrobasilar circulation, the PCA supplies the supratentorial structures of the cerebral hemisphere, mainly parietal, temporal, and occipital lobes. The PCA is connected to the internal carotid artery through the posterior communicating artery (Fig. 2, Fig. 5, Fig. 9, and Fig. 11) establishing the collateral pathway between the anterior (carotid) and posterior (vertebrobasilar) circulations, part of the circle of Willis (Fig. 11). The distal or the tip of the basilar artery and the two terminal posterior cerebral arteries encircle the midbrain. The posterior cerebral artery is subdivided into P1, P2, P3, and P4 segments. The P1 segment of PCA extends from the tip of the basilar artery to the junction with the posterior communicating artery. The P2 segment extends from the posterior communicating artery to the level of the posterior aspect of the midbrain. The P3 segment of the PCA is from the quadrigeminal plate of the midbrain to the level of the calcarine fissure. The segment of PCA within the calcarine fissure represents the P4 segment. The branches of the proximal segments of

Table 5
The Posterior Cerebral Artery

<i>Branches</i>
<i>Superficial cortical branches</i>
Temporal
Parietal occipital
Calcarine
<i>Callosal</i>
Splenial
<i>Choroidal</i>
Posterior medial choroidal
Posterior lateral choroidal
<i>Perforating</i>
Thalamoperforating
Thalamogeniculate
Peduncular
<i>Brain structures supplied</i>
Medial surface of the parietal lobe
Medial and inferior surface of the temporal lobe
Occipital lobe
Splenium of the corpus callosum
Choroid plexus of the third and lateral ventricles
Thalamus, hypothalamus
Posterior limb of the internal capsule and midbrain



Fig. 7. Selective left vertebral angiogram, frontal projection: 1, Large-caliber Left Vertebral Artery; 2, Smaller Caliber (anatomic variant) Right Vertebral Artery; 3, Basilar Artery; 4, Posterior Cerebral Arteries; 5, Posterior Inferior Cerebellar Artery; 6, Anterior Inferior Cerebellar Artery; 7, Superior Cerebellar Artery (duplicated); 8, Cortical Branches of the Posterior Cerebral Arteries.

the PCA are the perforating branches (thalamoperforating, thalamogeniculate), peduncular branches, choroid plexus (posterior medial choroidal artery, posterior lateral choroidal artery), anterior temporal cortical, middle temporal cortical and posterior temporal cortical branches, and splenial branches (Fig. 7, Fig. 8, Fig. 9, and Fig. 10).

The proximal P1 and P2 segments of the posterior cerebral arteries supply blood to the posterior thalamus, hypothalamus, internal capsule, midbrain, splenium of the corpus callosum, inferior surface of the temporal lobe, the choroid plexus of the third ventricle (medial posterior choroidal artery), and the choroid plexus of the lateral ventricle (lateral posterior choroidal artery).

The cortical branches of the distal posterior cerebral artery are the parietal occipital branch, calcarine branch, and anterior, middle, and posterior inferior temporal branches. The distal segment of the PCA supplies blood to the medial posterior third portion of the cerebral hemisphere, which includes part of the parietal lobe and most of the occipital and temporal lobes (Fig. 5, Fig. 7, Fig. 9, and Fig. 10).



Fig. 8. Selective left vertebral angiogram, frontal projection: 1, Right and Left Vertebral Arteries; 2, Basilar Artery; 3, Left Posterior Inferior Cerebellar Artery; 4, Right Anterior Inferior Cerebellar Artery; 5, Right and Left Superior Cerebellar Arteries; 6, Right and Left Posterior Cerebral Arteries.

2.2. Posterior (Vertebrobasilar) Circulation

2.2.1. THE VERTEBRAL ARTERIES (TABLE 6)

The vertebral arteries originate from the subclavian arteries and course anteriorly over the transverse processes (V1; extraosseous segment) and enter the transverse foramina of the sixth cervical vertebrae. The vertebral artery ascends within the transverse foramen from the sixth to the first cervical vertebra (V2; foraminial segment). The V3, or extraspinal segment of the vertebral artery exits the transverse foramen of the first cervical vertebra, and courses posterior and medial to enter the foramen magnum (V3; extraspinal segment). The two vertebral arteries penetrate the dura and the arachnoid membrane at the level of the foramen magnum to enter the intracranial cavity (V4; intradural segment). Both vertebral arteries course over the lateral surface of the lower medulla and come to lie between the clivus and the ventral surface of the medulla. The two vertebral arteries join to form the basilar artery in front of the brain stem at the pontomedullary junction (Fig. 7, Fig. 8, Fig. 9, and Fig. 10).

Anterior and posterior meningeal branches of the vertebral artery supply the meninges of the posterior

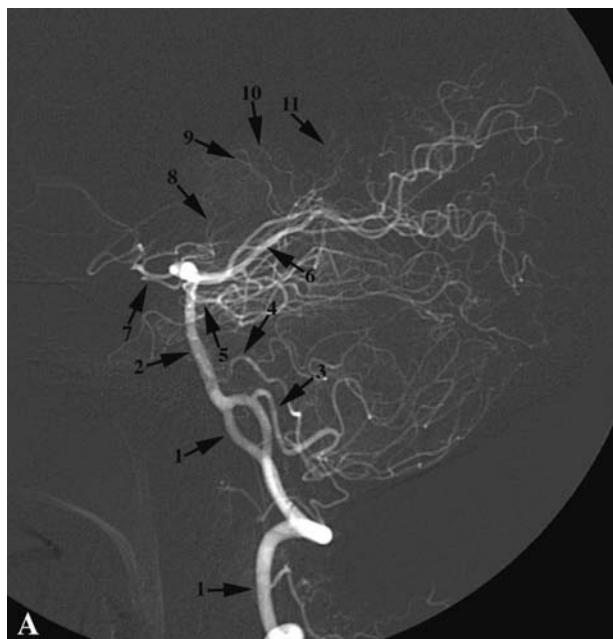


Fig. 9. Selective vertebral angiogram, lateral projection: 1, Vertebral Arteries; 2, Basilar Artery; 3, Posterior Inferior Cerebellar Artery; 4, Anterior Inferior Cerebellar Artery; 5, Superior Cerebellar Artery; 6, Posterior Cerebral Arteries; 7, Posterior Communicating Arteries; 8, Thalamoperforating, Thalamogeniculate, and Peduncular perforating branches; 9, Medial Posterior Choroidal Arteries; 10, Lateral Posterior Choroidal Arteries; 11, Splenial Branches.

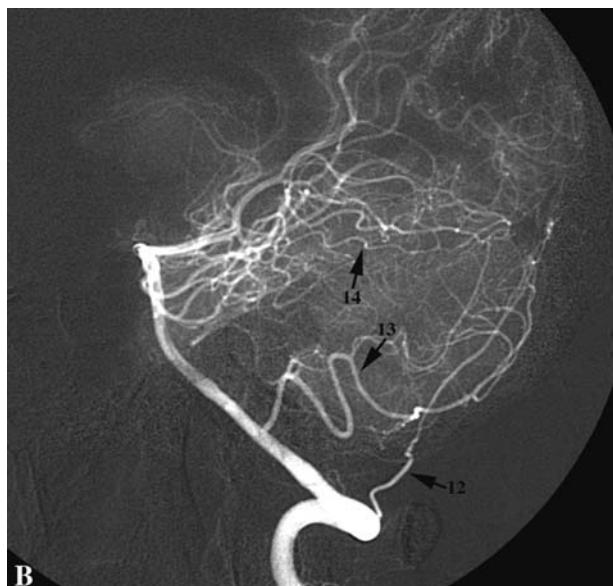


Fig. 10. Selective vertebral angiogram, lateral projection: 12, Posterior Meningeal Branch; 13, Tonsilla-Hemispheric Branches of Posterior Inferior Cerebellar Artery; 14, Hemispheric Branches of Superior Cerebellar Arteries.

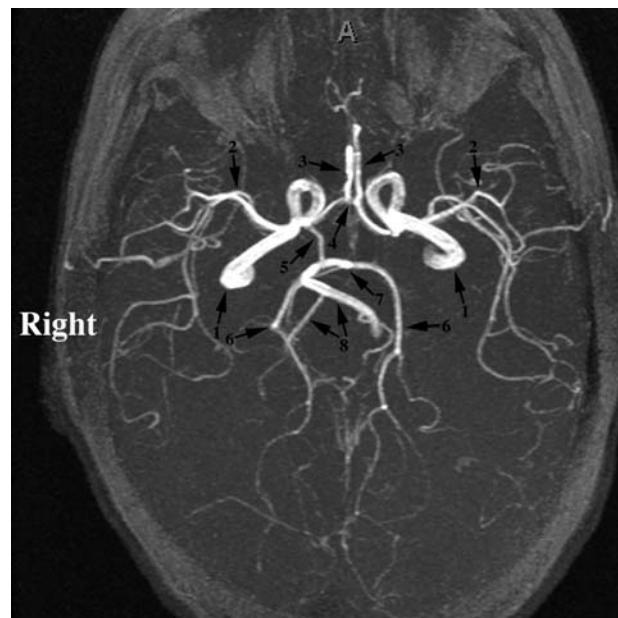


Fig. 11. Axial view of three-dimensional time-of-flight magnetic resonance angiography showing circle of Willis: 1, Internal Carotid Artery; 2, Middle Cerebral Artery; 3, Anterior Cerebral Artery; 4, Anterior Communicating Artery; 5, Posterior Communicating Artery; 6, Posterior Cerebral Artery; 7, Basilar Artery; 8, Right and Left Vertebral Arteries.

Table 6
Intracranial Vertebral Artery

Branches

- Posterior inferior cerebellar artery
- Anterior spinal artery
- Posterior spinal arteries
- Meningeal arteries
- Perforating arteries

Blood supply

- Medulla oblongata
- Upper cervical spinal cord
- Inferior surface of the cerebellar hemisphere
- Inferior vermis

cranial fossa. The anterior spinal artery (Fig. 8) and the paired posterior spinal arteries begin their courses within the intracranial cavity as branches of the vertebral arteries or posterior inferior cerebellar arteries and supply the spinal cord along with numerous radiculomedullary arteries within the spine. The spinal branches and the small number of perforating branches arising from the vertebral artery supply the lower medulla, upper cervical spinal cord, and the inferior cerebellar peduncles.

The posterior inferior cerebellar artery (PICA) is the largest branch of the vertebral artery (Fig. 7, Fig. 8, Fig. 9, and Fig. 10) and supplies the lateral and posterior aspect of the medulla, the choroid plexus of the fourth ventricle, the cerebellar tonsils, the inferior cerebellar vennis, and the inferior aspects of the cerebellar hemispheres.

2.2.2. THE BASILAR ARTERY (TABLE 7)

The basilar artery is formed by the union of the two vertebral arteries at the level of the pontomedullary junction and the origin of the sixth cranial nerves (Fig. 7, Fig. 8, Fig. 9, and Fig. 10). The basilar artery courses over the ventral surface of the pons behind the clivus and terminates in front of the midbrain into two posterior cerebral arteries at the level of the origin of the third cranial nerves posterior to the dorsum sella. The branches of the basilar artery are anterior inferior cerebellar arteries (AICA), superior cerebellar arteries (SCA) (Fig. 7, Fig. 8, Fig. 9, and Fig. 10), pontine median and paramedian perforating branches, and labyrinthine (internal auditory) artery. The median pontine perforating, paramedian pontine perforating, and lateral circumferential branches form the numerous perforating branches of the basilar artery and supply the pons, midbrain, and cerebellar peduncles. The median and paramedian perforating branches are associated with the same disease process as the striate branches of the anterior cerebral and middle cerebral arteries in chronic hypertension with lacunar infarcts.

The anterior inferior cerebellar artery supplies the anterior or ventral surface of the cerebellar hemisphere, pons, and cerebellar peduncle (Fig. 7 and Fig. 8).

Table 7
Basilar Artery

<i>Branches</i>
Anterior inferior cerebellar artery
Pontine perforating
Labyrinthine (internal auditory)
Superior cerebellar arteries
Posterior cerebral arteries
<i>Blood supply</i>
Brain stem, mainly pons
Anterior surface of the cerebellum
Superior surface of the cerebellum
Superior vennis
Midbrain
Part of the thalamus
Labyrinth of inner ear

The superior cerebellar artery arises just proximal to the bifurcation of the basilar artery and supplies the superior and lateral surface of the cerebellar hemispheres, the superior cerebellar peduncle, pons, and the superior cerebellar vermis (Fig. 7, Fig. 8, Fig. 9, and Fig. 10).

The tip of the basilar artery at the level of the bifurcation into the two posterior cerebral arteries also gives rise to small thalamoperforating and thalamogeniculate branches, which supply the thalamus and the internal capsules (Fig. 9).

All the arteries of the posterior or the vertebrobasilar circulation supply the contents of the infratentorial posterior cranial fossa, except the two posterior cerebral arteries, which course through the tentorial incisura into the supratentorial compartment supplying the cerebral hemispheres.

2.2.3. LEPTOMENINGEAL OR PIAL VESSELS

Pial vessels describe the small branches of the circle of Willis arteries that course through the surface of the brain for various distances before penetrating into the brain parenchyma. Numerous arterial anastomoses exist among these leptomeningeal or pial branches. Two types are found: large-diameter end-to-end anastomoses that connect branches from two different arterial stems (e.g., branches of the middle and anterior cerebral arteries) and extremely-small-diameter anastomoses connecting branches from the same or a different parent artery. The diameter of the largest anastomoses joining arterioles end-to-end varies from 25 to 90 μm . The average diameter of the small, straight anastomoses is 10 μm .

2.2.4. INTRAPARENCHYMAL OR PENETRATING ARTERIES

Cortical arteries divide into small pial vessels before penetrating the cortex. Each penetrating cortical artery forms a vascular palisade that supplies the respective capillary bed. Major vessels supplying the cortex enter the gyral surface at a perpendicular angle; those with the largest caliber and longest course supply the deepest cortical layers. Anastomoses forming a continuous horizontal layer appear in layer three of the cortex among the large pyramidal cells, but the greatest number of these connecting vessels is visible in layers four and five, and few are visible in layer six of the cortex.

Central arteries at each gyrus always have a large diameter (260 to 280 μm) at their point of origin. Peripheral arteries have an average diameter of 150 to 180 μm . On the cortical surface, all arterioles measuring 50 μm or less penetrate the cortex or anastomose with neighboring ones. Penetrating vessels into the cerebral

cortex are short arterioles (<100 µm in diameter) devoid of continuous internal elastic lamina and having a tunica media composed of one or two layers of smooth-muscle cells. Most penetrating arterioles have a diameter of approximately 40 µm.

Some of the *large penetrating arteries* are branches that originate directly from the trunk of a large vessel, such as the middle cerebral artery and the basilar artery. These branches supply the basal ganglia and the thalamic nuclei, respectively. Penetrating arteries at these sites are long, muscular vessels (100 to 400 µm in internal diameter) endowed with internal elastic lamina and three or four layers of smooth-muscle fibers in the tunica media.

One of the few groups of vessels supplying gray-matter structures that lack anatomic anastomoses among themselves are the *lenticulostriate branches* originating from the main trunk of the middle cerebral artery. The lenticulostriate vessels have been implicated as being the source of the most common type of non-traumatic intracerebral hemorrhage—hypertensive hemorrhage.

Forty percent of the total vascular resistance in the CNS can be traced to the penetrating parenchyma arteries that are less than 200 µm in diameter. Chronic arterial hypertension increases the resistance in these small arteries, protecting the microvasculature (e.g., arterioles, capillaries, venules) from the effects of sustained high blood pressure. The vascular resistance in extracranial organs is primarily a function of arterioles.

Blood flow to subcortical white matter fibers, also called U-fibers, is supplied by arteries and arterioles, but the deeper white-matter structures (e.g., centrum semiovale) are supplied only by nonanastomosing, long radial arteries.

Long transcortical vessels traverse the cortex without branching and, on entering the subjacent white matter, form a cascade of vessels that terminates in a periventricular plexus. The long, penetrating radial arteries that supply the cerebral hemispheric white matter do not interconnect with one another. The name *terminal arteries* has been applied to these and other nonanastomosing vessels.

2.3. The Structure of Intradural Arteries Is Different from That of Extracranial Arteries

The histologic structure of the arteries supplying the brain changes as these vessels penetrate the dura mater. The elastic fibers, which in extracranial arteries are distributed throughout the entire width of the arterial wall, condense into a subendothelial elastic lamina after the arteries penetrate the dura mater (Fig. 12).

The thickness of the tunica media is decreased in intracranial vessels compared with arteries of the same caliber located outside the skull.

Intradural arteries and veins are bathed in CSF, even after the arteries penetrate the cerebral parenchyma; this is made possible by perivascular sheath-like extensions of the subarachnoid space into the brain parenchyma. The anatomic, perivascular spaces within the brain that contain the brain vessels in the subarachnoid space are known as the Virchow-Robin spaces. The arteries and arterioles in the Virchow-Robin spaces lack tunica adventitia; in these vessels, the muscular tunica media is surrounded by a single-cell layer of leptomeningeal origin. The Virchow-Robin space disappears when the *glia limitans*, a subpial structure formed mainly by the end-feet of astrocytes and attached to the pial membrane, fuses with the basal lamina of the smallest arterioles and capillaries.

Intracranial arteries frequently exhibit *medial defects* or interruptions in the continuity of the smooth-muscle layers at bifurcation sites (e.g., distal carina). These are the same sites in which saccular aneurysms develop in persons with inherited disorders of connective tissue metabolism.

At the branching sites of the intracranial arteries such as proximal carina, there are *intimal cushions* or sites where well-demarcated intraluminal protrusions are formed by subendothelial aggregates of smooth-muscle fibers. These intimal cushions become more apparent with increasing age, but their functional significance has not been elucidated. Normal intracranial arteries in persons younger than 20 years of age probably lack *vasa vasorum*; aging and diseases associated with this process, such as atherosclerosis, may induce the development of a few vessels in the tunica adventitia of intradural arteries.

2.4. Three Types of Nerve Fibers Have Endings on the Walls of Large Intracranial Arteries

2.4.1. PAIN-SENSITIVE FIBERS

Pain-sensitive fibers were first demonstrated in humans by electrical stimulation of arteries attached to the dura (e.g., the branches of the external carotid artery) and arterial branches of the internal carotid artery located within the subarachnoid space. Substance P is the main tachykinin involved in the transmission of nociceptive information. Using immunohistochemical methods, substance P has been identified on the tunica adventitia of large intracranial vessels. Many intracranial and extracranial blood vessels that supply the brain are surrounded by axonal

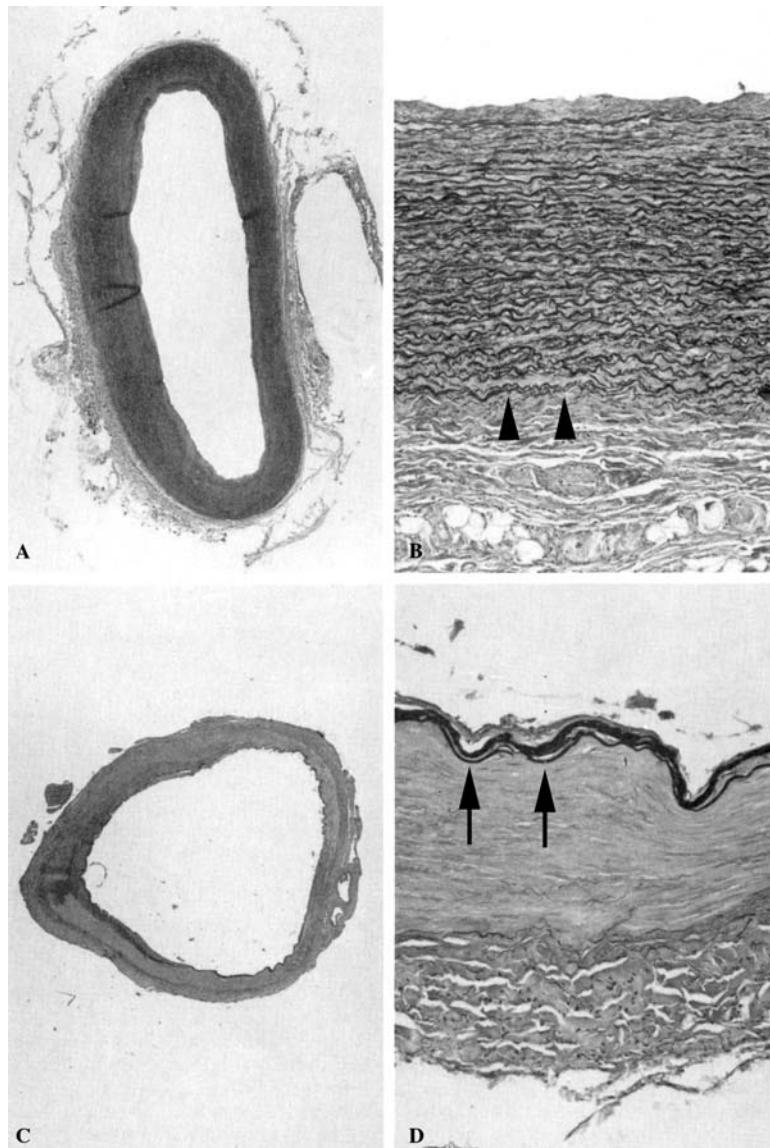


Fig. 12. (A, B) Normal common carotid artery. (C, D) Normal intradural portion of the internal carotid artery. The black wavy line (arrowheads) corresponds with elastic fibers. The elastic fibers condense into the subendothelial elastic lamina (arrows) after the arteries penetrate the dura mater.

terminals originating from the trigeminal and the upper dorsal root ganglia. The densest network of these fibers exists on the tunica adventitia of the major arterial branches as they emerge from the circle of Willis.

The caudal portion of the basilar artery and both vertebral arteries and their tributaries are innervated by fibers originating in the upper cervical dorsal-root ganglia. The central projections of the fibers originating from the trigeminovascular system are not fully understood.

Bleeding in the subarachnoid space is one of the best-known causes of severe headache; this is a unique response limited to the intracranial vessels. The

trigeminovascular system of nerve fibers around arteries, which includes substance P-dependent fibers, is believed to be intimately involved in the mechanism of pain and migraine headaches.

2.4.2. SYMPATHETIC FIBERS

Sympathetic fibers supplying large intracranial vessels originate primarily from the superior cervical ganglion, the middle cervical sympathetic ganglion, and the sympathetic ganglion. In addition to norepinephrine, sympathetic nerve terminals also secrete the vasoconstrictor neuropeptide Y. Sympathetic fibers are not involved in the regulation of the cerebral

blood flow, except that sympathetic stimulation blunts anticipated rises in cerebral blood flow during severe arterial hypertension. The most important role of the sympathetic innervation in intracranial arteries is to protect small arteries from the effects of blood pressure surges within the physiologic range.

2.4.3. PARASYMPATHETIC FIBERS

Parasympathetic fibers to the cerebral blood vessels originate mainly from sphenopalatine, otic, and associated miniganglia. The most dense network of fibers exists around the proximal segments of arteries branching out of the circle of Willis. In addition to cholinergic fibers, parasympathetic nerve endings contain vasoactive intestinal polypeptide (VIP). Nitric oxide synthase (NOS) has been colocalized in the same fibers that contain VIP. NOS may be the same as the endothelium-dependent relaxing factor. Cholinergic mechanisms have a minimal influence in the control of the normal cerebral blood flow.

None of the cerebral perivascular networks of nerve fibers plays a significant role in the normal autoregulation of blood flow to the brain. Autoregulation is primarily the result of myogenic responses to changes in blood pressure or in neuronal metabolism. Sensory parasympathetic as well as sympathetic nerves contribute to the cerebral blood flow regulation and preserve the integrity of the blood-vessel wall only in pathologic conditions such as sustained hypertension and chronic hypotension.

3. COLLATERAL CIRCULATION (TABLE 8)

A system of abundant collateral circulation protects the brain from isolated arterial occlusion or stenosis. Terminal, small pial cortical branches of the cerebral arteries anastomose with each other across a vulnerable watershed or border zone. This abundant end-to-end anastomosis forms an extensive arterial network over the surface of the brain, which facilitates the pial collateral circulation and acts as a protective mechanism against focal disruption of the blood flow to the brain.

This system of collateral or alternate circulation allows distal branches of an occluded artery to fill in a retrograde fashion through the end-to-end anastomoses that connect neighboring vessels and to compensate for the changes in blood flow.

The anterior (e.g., carotid) circulation and the posterior (e.g., vertebrobasilar) circulation are connected by the posterior communicating arteries that connect the internal carotid artery with the ipsilateral posterior cerebral artery. Arterial connection between the right and

Table 8
Collateral Circulation

<i>Circle of Willis</i>	An anastomotic arterial ring at the base of the brain formed by:
	Right and left internal carotid arteries
	Right and left anterior cerebral arteries
	Right and left posterior cerebral arteries
	Anterior communicating artery
	Posterior communicating arteries
<i>Anterior communicating artery</i>	Connects right and left anterior (carotid) circulation
<i>Posterior communicating artery</i>	Connects the anterior (carotid circulation) to the posterior (vertebrobasilar) circulation
<i>Pial collateral anastomoses</i>	Connect the terminal pial cortical branches of the cerebral arteries across a vulnerable water shed or border zone
<i>Pial leptomeningeal anastomoses</i>	Pial leptomeningeal anastomoses connect pial branches of the cerebral arteries to the meningeal branches of the external carotid, internal carotid, and vertebral arteries
<i>Intraorbital anastomoses</i>	Between the branches of the external carotid artery and the branches of the ophthalmic artery within orbit

left anterior (carotid) circulation across the midline is provided by the anterior communicating artery. Credit for the correct description of the arterial anatomic network located at the base of the brain is given to Thomas Willis, and the *circle of Willis* (Fig. 11) designates this arterial network, which provides the best potential collateral flow of blood in vascular occlusive disease.

These anastomotic connections are significant because of the collateral circulation they provide. In many instances, local circulatory abnormalities created by occluding a single artery at points proximal to the circle of Willis can be adequately compensated through the collateral circulation.

The circle of Willis and its feeding branches constitute a symmetric structure in only about 40% of adults examined postmortem. A significant number of anatomic variations, which in most cases reflect the persistence of embryonal or fetal vascular patterns, are found in about 60%. Among the most common variations in the anatomy of the circle of Willis are hypoplasia of one vertebral artery and of the contralateral anterior cerebral artery or a posterior cerebral

artery originating from the internal carotid artery instead of the basilar artery.

Anastomoses among branches of the internal and external carotid arteries exist through the ophthalmic artery (e.g., the large branch of the internal carotid artery) and its end-to-end connections with facial branches of the ipsilateral external carotid artery. There are extensive collateral anastomoses between pial meningeal branches of the internal carotid and meningeal branches of the external carotid artery.

4. CAPILLARIES

The perivascular sheath of CSF surrounding penetrating arteries and arterioles (perivascular space or Virchow-Robin space) disappears where the glia limitans merges with the basal lamina of the brain capillaries. These vessels, which in humans are 4 to 7 μm in diameter, are composed of one endothelial-cell layer, resting on a basal lamina that completely encircles a pericyte. Pericytes do not form a continuous layer around the endothelial-cell layer; individual pericytes are found at infrequent intervals on the luminal side of the capillary wall. Encircling the basal lamina of the endothelial cell or the pericyte are numerous processes of astrocytes joined to one another by *gap junctions*.

4.1. The Blood-Brain Barrier Refers to a Complex Array of Physical, Metabolic, and Transport Properties of the Capillary Endothelium

The blood-brain barrier is a complex anatomic or mechanical, physiologic and osmotic barrier protecting the brain. Circulating macromolecules, such as globulins and albumin, do not cross the endothelial lining of brain capillaries. This contrasts with the ready escape of circulating macromolecules that normally occurs in most extracranial tissues. The original description of the blood-brain barrier is attributed to Ehrlich who, in 1885, observed that intravenous injections of Evans blue, a dye that circulates bound to albumin, result in the diffuse distribution of the dye to almost every organ and tissue except the brain and spinal cord.

The concept of a blood-brain barrier describes the inability of circulating macromolecules to enter the extracellular space or interstitial fluid of the brain and spinal cord. The mechanical component of the barrier has been traced primarily to structural characteristics of the endothelial capillary lining of the brain and spinal cord that are lacking in the endothelial

lining of capillaries in other organs. A first important feature is that endothelial cells lining capillaries and venules in the CNS are joined at the luminal portion by *zonulae occludentes* or pentalaminar structures that represent the fusion of the outermost layers of two apposing endothelial-cell membranes (Fig. 13). The second factor preventing the escape of circulating macromolecules in the brain is the paucity of endocytotic pits in the endothelium of most vessels in the CNS. In contrast, the endothelial lining of capillaries and venules in extraneuronal tissues has abundant endocytotic pits and sizable gaps or fenestrae through which circulating particles such as 40-kDa horseradish peroxidase or 445-kDa apo ferritin readily escape into the surrounding interstitial fluids.

Cerebral endothelium may become abnormally permeable to circulating macromolecules by several mechanisms: enhanced transcytosis or transport of molecules across the endothelial cytoplasm by means of endothelial vesicles; separation of the endothelial junctions; formation of tubular channels by fusion of endothelial vesicles; and loss of the negative charge

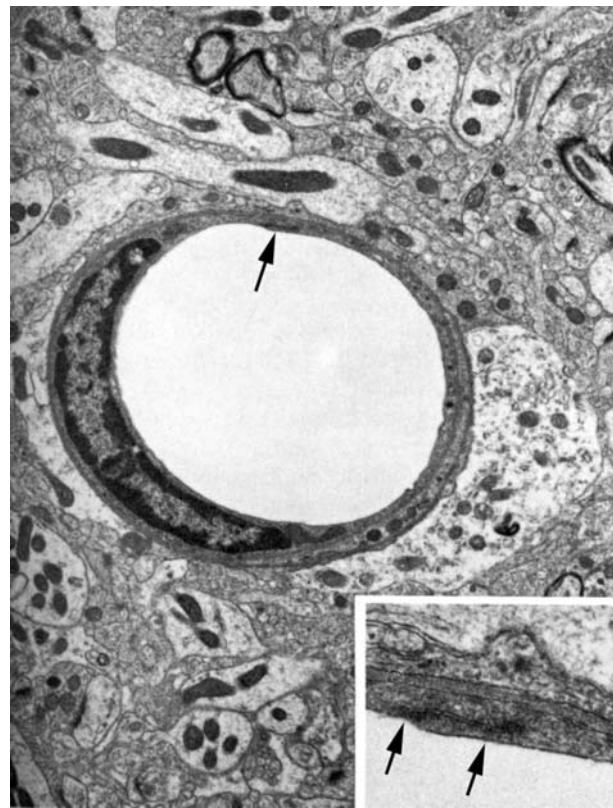


Fig. 13. Normal rat brain capillary (original magnification $\times 7000$). The inset shows a close-up view of the capillary wall to demonstrate a tight junction (arrows) (original magnification $\times 32,200$).

on the endothelial surface, particularly loss of the terminal sialic group on the luminal side of the endothelial plasma membrane.

4.2. Capillaries at the Circumventricular Organs Are Permeable to Circulating Macromolecules

The circumventricular organs are seven small, well-circumscribed areas located at the ependymal border of the third and fourth ventricles (Fig. 14), where capillaries are permeable to hydrophilic solutes. These sites are the pineal body, median eminence, neurohypophyseal-hypothalamic axis, subcommissural organ, area postrema, subfornical organ, and organum vasculosum of laminae terminalis.

The circumventricular organs are endowed with permeable capillaries that have fenestrated endothelium, with the exception of the subcommissural organ. The functions of the circumventricular organs are uncertain, although some investigators suggest that macromolecular permeability at these sites may be related to the involvement of the respective neuronal groups in the regulation of neuroendocrine functions.

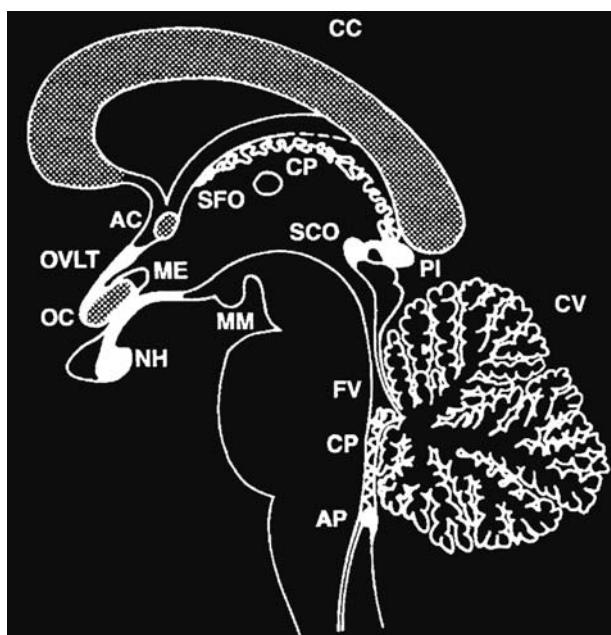


Fig. 14. Midline sagittal schematic drawing of the brain showing circumventricular organs (dark shaded structures): NH, neurohypophysis; ME, median eminence; OVLT, organum vasculosum of lamina terminales; SFO, subfornical organ; PI, pineal gland or body; SCO, subcommissural organ; AP, area postrema; CP, choroid plexus; OC, optic chiasm; AC, anterior commissure; CC, corpus callosum (lightly shaded areas).

4.3. Immune and Inflammatory Mediators Play an Important Role in a Variety of Pathophysiologic Pathways Such as in Cerebral Ischemia

After cerebral ischemia, leukocytes—through their initial interactions with the microvascular endothelium—play an important role in the development of the infarct. Within hours of the onset of ischemia, polymorphonuclear (PMN) leukocytes accumulate and obstruct the microvasculature, then enter the parenchyma, followed by cells of the monocyte/macrophage lineage. Adhesion to the endothelium and transmigration through the vessel wall are influenced by inflammatory mediators, including cytokines. Upon activation, under conditions of ischemia/reperfusion, PMN leukocytes and the endothelium generate free radicals and proteases that contribute to microvascular and tissue injury.

4.3.1. ADHESION RECEPTORS

Adhesion receptors that mediate cell-cell and cell-matrix interactions in the cerebrovasculature belong to three families: selectins, integrins, and immunoglobulin-related receptors. Their inhibition may modulate cellular inflammation and reduce infarction. The access of PMN and other leukocytes to perivascular cells in the developing infarction requires their direct contact with the microvascular endothelium. After middle cerebral artery occlusion (and reperfusion), PMN leukocytes contribute to microvascular obstruction and edema formation during their adherence to the endothelium. Adherence and transmigration of PMN leukocytes through the postcapillary endothelium involve the sequential interaction of P-selectin, intercellular adhesion molecule (ICAM-1), and E-selectin. The selectin family consists of P-selectin found on platelets and endothelial cells, E-selectin (endothelial cells), and L-selectin (leukocytes). P-selectin on endothelial cells and platelets mediates their interaction with granulocytes and monocytes. Integrins are heterodimeric adhesion molecules with a ubiquitous distribution. The adhesion properties of certain integrins are central to leukocyte transmigration. Firm adhesion is mediated by the interaction of granulocyte β_2 -integrins with endothelial cell ICAM-1 (integrin $\alpha_M \beta_2$ MAC-1), or endothelial cell ICAM-1 and ICAM-2 (integrin $\alpha_L \beta_2$, LFA-1).

4.3.2. CYTOKINES

Ischemic cerebral tissues generate cytokines, superoxide free radicals, biogenic amines, and thrombin. These are stimulators for endothelial cells, granulocyte,

and platelet activation and adhesion-receptor expression. Cytokines connect the pathophysiologic mechanisms of inflammation and ischemia. Tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), IL-6, and IL-8, together with monocyte chemoattraction protein-1 (MCP-1) are known protagonists in experimental cerebral ischemia. Cytokine release is important for the transition from focal cerebral ischemia to inflammation.

4.4. Complement Activation

Together with the acute inflammatory response that occurs during cerebral ischemia, complement components are activated, which contributes to cell injury. Activated complement contributes to the extension of the injury by promoting neutrophil accumulation through several mechanisms. In addition, C3a and C5a lead to histamine release, and the C5b-9 complex results in loss of cell-membrane integrity. One possible therapeutic strategy in focal cerebral ischemia would be the blockade of the complement cascade.

5. INTRACRANIAL VENOUS SYSTEM (TABLE 9)

Deep cerebral and superficial cortical veins drain most of the intracranial structures and the brain. The deep and the superficial cerebral veins empty blood into the intracranial dural venous sinuses, which are unique valveless venous channels lined by endothelium and are enclosed between the outer (periosteal) and inner (meningeal) layers of dura. All dural venous sinuses finally converge and drain blood into the right and left internal jugular veins (Fig. 15). The internal jugular veins connect with their respective subclavian veins to form innominate veins in the neck. The right and left innominate veins subsequently unite to form the superior vena cava, which drains into the right atrium of the heart. Extensive collateral pathways

Table 9
Intracranial Venous System

Deep cerebral venous system

Drains the anastomotic structures of the cerebral hemispheres near the midline, the deep cerebral structures such as the deep white matter, thalamus, basal ganglia, and the upper brain stem

Superficial venous system

Drains the cerebral cortex and adjacent white matter

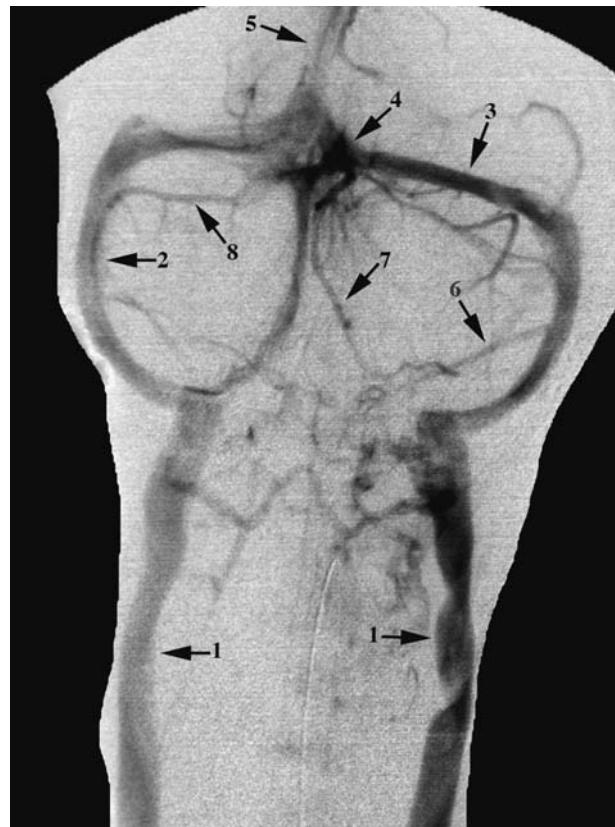


Fig. 15 Venous phase of vertebral angiogram, frontal projection: 1, Internal Jugular Vein; 2, Sigmoid Sinus; 3, Transverse Sinus; 4, Confluence of Sinus (torcular herophili); 5, Superior Sagittal Sinus; 6, Superior Petrosal Sinus; 7, Inferior Vermian Vein; 8, Cerebellar Hemispheric Veins.

exist between superficial cortical veins, the deep cerebral veins, and the dural venous sinuses.

5.1. The Deep Venous System (Table 10)

5.1.1. THE DEEP CEREBRAL VENOUS SYSTEM PRIMARILY DRAINS VEINS ORIGINATING IN MIDLINE STRUCTURES

The veins of the deep cerebral white matter, the basal ganglia, and thalamus drain centrally and centrifugally into the deep venous system, which ultimately drains into the subependymal veins that are located in the subependymal region around the lateral ventricles. All the subependymal veins drain into the paired internal cerebral veins near the midline and are located in the roof of the third ventricle (Fig. 16, Fig. 17, and Fig. 18). The basal veins of Rosenthal (Fig. 17 and Fig. 18) begin near the anterior perforated substance near the sylvian fossa and are formed from confluence of the deep middle cerebral, insular, and striate veins that drain the insular cortex, the corpus striatum, and the deep basal ganglia. The basal vein of Rosenthal

Table 10
The Deep Venous System

Paired internal cerebral veins

Basal vein of Rosenthal

Great cerebral vein of Galen

Brain structures

Deep white matter of the cerebral hemispheres

Corpus callosum

Thalamus

Basal ganglia

Internal capsule

Choroid plexus of the third and lateral ventricles

Subependymal regions of the lateral and upper third ventricles

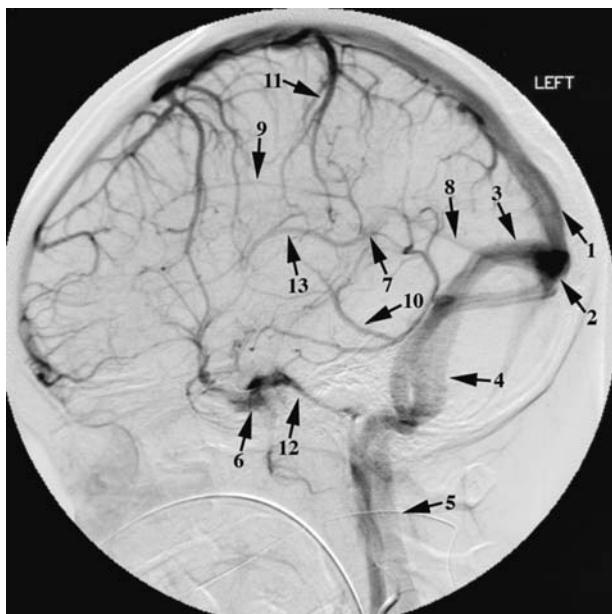


Fig. 16. Early venous phase of carotid angiogram, lateral projection: 1, Superior Sagittal Sinus; 2, Confluence of Sinus or Torcular Herophili; 3, Transverse Sinus; 4, Sigmoid Sinus; 5, Internal Jugular Vein; 6, Cavernous Sinus; 7, Vein of Galen; 8, Straight Sinus; 9, Inferior Sagittal Sinus; 10, Vein of Labbe; 11, Superficial Cortical Veins—Vein of Trolard; 12, Inferior Petrosal Sinus; 13, Internal Cerebral Veins.

courses posterior between the cerebral peduncles and the medial surface of the temporal lobes and ultimately joins the vein of Galen in the midline (Fig. 16, Fig. 17, and Fig. 18). The vein of Galen empties blood into the straight sinus at the junction of the inferior sagittal sinus.

The basal vein of Rosenthal drains blood from the insular cortex, basal ganglia, medial temporal lobe,



Fig. 17. Late venous phase of the internal carotid angiogram: 1, Basal Vein of Rosenthal; 2, Internal Cerebral Vein; 3, Vein of Galen; 4, Straight Sinus; arrowheads, subependymal tributaries of the internal cerebral vein.

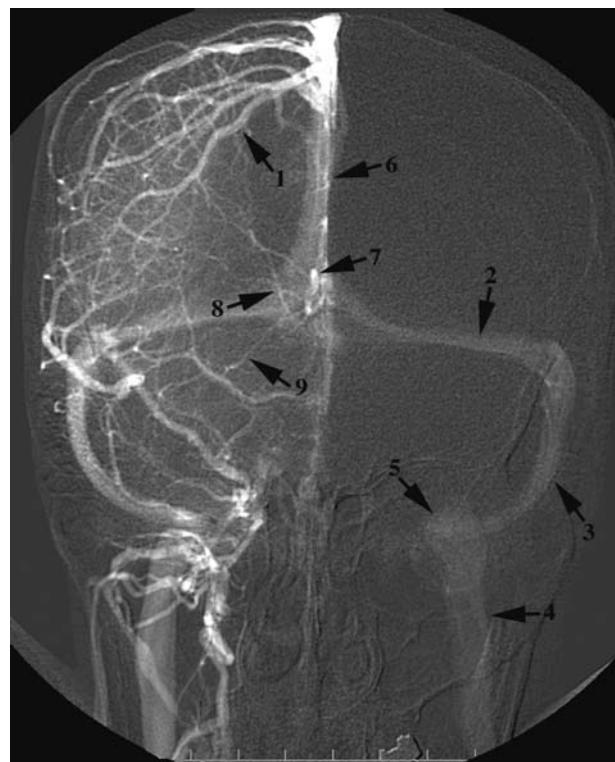


Fig. 18. Frontal projection of the late phase of the left carotid angiogram: 1, Superficial Cortical Veins draining into the Superior Sagittal Sinus; 2, Transverse Sinus; 3, Sigmoid Sinus; 4, Internal Jugular Vein; 5, Jugular Bulb; 6, Superior Sagittal Sinus; 7, Internal Cerebral Vein; 8, Thalamostriate Vein; 9, Basal Vein of Rosenthal.

midbrain, thalamus, choroid plexus of the temporal horn of the lateral ventricle, subependymal region, and the deep white matter of the temporal lobe.

The internal cerebral vein drains blood from the choroid plexus of the third ventricle, choroid plexus of the lateral ventricles, basal ganglia, internal capsule, thalamus, subependymal region of the lateral ventricles, and the deep white matter of the cerebral hemispheres (Fig. 16, Fig. 17, and Fig. 18).

The system of the deep cerebral veins includes several veins that drain anatomic structures of the cerebral hemispheres located near the midline; most of these veins converge into the great vein of Galen. The paired midline internal cerebral vein unite to form the great cerebral vein of Galen. Veins from the deep cerebral structures, such as the thalamus, join the subependymal veins that drain into the internal cerebral vein. The basal vein of Rosenthal, the internal occipital vein, and other vessels also contribute to the great vein of Galen. The great cerebral vein of Galen, which is 2.0 cm long, is close to the pineal body, quadrigeminal plate, and dorsum of the superior cerebellar vermis. The deep middle cerebral veins drain the insular cortex and portions of the adjacent opercular surface, and these veins usually receive lenticulostriate veins that drain the inferior portion of the basal ganglia. The thalamostriate, anterior caudate, septal, and midatrial veins are tributaries of the internal cerebral vein.

The vein of Galen also drains anatomic structure related to the optic chiasm, uncinate gyrus, parahippocampal gyrus, portions of the ventricular temporal horn, and upper brain stem. Venous flow originating from these sites enters the vein of Galen through the basal veins of Rosenthal. The basal vein of Rosenthal is lateral to the optic chiasm and courses around the cerebral peduncles to terminate into the great vein of Galen. Shortly after its origin, the basal vein is covered by the uncus and the parahippocampal gyrus before it circles around the cerebral peduncle.

The vein of Galen and the inferior sagittal sinus—a venous structure running parallel to the dorsal surface of the corpus callosum within the free edge of falx cerebri—join one another to form the straight sinus, which occupies a midline position in the tentorium cerebelli (Fig. 16, Fig. 17, and Fig. 18). The inferior sagittal sinus courses above the corpus callosum along the free edge of the falx cerebri (Fig. 16). This sinus receives numerous veins that drain the roof of the corpus callosum, the cingulate gyrus, and adjacent structures of the cerebral hemisphere. Most of the superior cerebellar veins drain into the straight

sinus. The straight sinus ends in the torcular herophili, a dura mater structure located at the site of the internal occipital protuberance (Fig. 15 and Fig. 16). The torcular herophili is the site of confluence of the straight sinus, right and left transverse sinuses, and the superior sagittal sinus, which sometimes remain separate from one another. The straight sinus usually drains into the left transverse sinus, and the superior sagittal sinus continues into the right transverse sinus.

The transverse sinuses drain into the sigmoid sinuses, which originate at the posterior petrous portion of the temporal bone. The sigmoid sinus ends in the jugular bulb within the jugular foramen, where it continues into the internal jugular vein. The superior petrosal sinus usually drains into the proximal portion of the sigmoid sinus. This sinus also receives veins from the cerebellum, the lateral pons, and the medulla. The two internal jugular veins with respective innominate veins converge to form the superior vena cava.

5.2. Superficial Cerebral Veins (Table 11)

5.2.1. SUPERFICIAL CEREBRAL VEINS DRAIN INTO EITHER THE SUPERIOR SAGITTAL SINUS, TRANSVERSE SINUSES, OR SIGMOID SINUSES

The numerous superficial cortical veins drain the cortex and the adjacent white matter and lie within the cerebral sulci (Fig. 16 and Fig. 18). Most of the superficial cortical veins are unnamed because of their variable appearance. The superficial middle cerebral vein, the anastomotic vein of Trolard (Fig. 16), and anastomotic vein of Labbe (Fig. 16 and Fig. 17) are consistently seen. The superficial middle cerebral vein is located along the sylvian fissure. The anastomotic vein of Trolard, located along the cortical surface of the anterior parietal lobe, and the vein of Labbe courses over the cortical surface of the temporal lobe. A rich network of collateral anastomosis

Table 11
Superficial Venous System

Anastomotic vein of Labbe
Anastomotic vein of Trolard
Superficial middle cerebral vein
Numerous unnamed superficial cortical veins
<i>Brain structures drained</i>

Cortex of the cerebral hemisphere
Rich network of collateral channels exist between the numerous superficial cortical veins

exists between the numerous superficial cortical veins and the deep cerebral veins that become more visible after venous occlusive disease.

Superficial cerebral veins designate the network of venous channels that are visible on the surface of each cerebral hemisphere. Superficial cerebral veins coalesce on the pial surface and convey blood from the outer 1 or 2 cm of the cortex and underlying superficial white matter. The superficial veins are divided into *superior* veins that drain into the superior sagittal sinus and *inferior* veins whose flow is directed into the transverse sinus; a *middle* group of vessels may drain superiorly or inferiorly. All the superficial veins normally empty into one or more of the major dural sinuses. The inferior superficial veins drain territories located primarily on the ventral surface of the temporal and occipital lobes.

The main draining avenue for most superficial cerebral veins, the superior sagittal sinus, originates at the foramen cecum of the frontal bone, courses anteroposteriorly along the midline of the dura mater, and ends in the torcular herophili (Fig. 15 and Fig. 16).

Most superficial veins follow their course through the subarachnoid space over the surface of the various cerebral gyri, and the superior superficial veins usually converge on each side to form four to six large veins that, after piercing the arachnoid membrane, terminate in the walls of the superior sagittal sinus. The short segments of these veins located in the subdural space are designated as superficial cortical *bridging* veins. In most instances of subdural bleeding, the hemorrhage is believed to originate from tears in the bridging segment of the superficial cerebral veins.

5.3. Posterior Fossa (Infratentorial) Veins (Table 12)

The veins of the posterior fossa are grouped under superior (Galenic) group, anterior (petrosal) group, and posterior (tentorial) group. The precentral cerebellar vein, superior vermicular vein, and anterior pontomesencephalic veins form the superior Galenic group and drain the superior surface of the cerebellar hemispheres, superior vermis, upper pons, and midbrain (Fig. 15 and Fig. 19). The anterior (petrosal) group of veins includes petrosal veins that drain the ventral surface of the pons, medulla, and the ventral surface of the cerebellar hemispheres. The petrosal veins subsequently drain into the superior petrosal sinus. The posterior (tentorial) group of veins includes inferior vermicular veins that drain the inferior surface of the cerebellar hemispheres and the inferior vermis and dorsal surface

Table 12
Posterior Fossa Venous System

Superior (Galenic) group
Anterior (petrosal) group
Posterior (tentorial) group
<i>Brain structures drained</i>
Brain stem
Cerebellar hemispheres
Cerebellar vermis

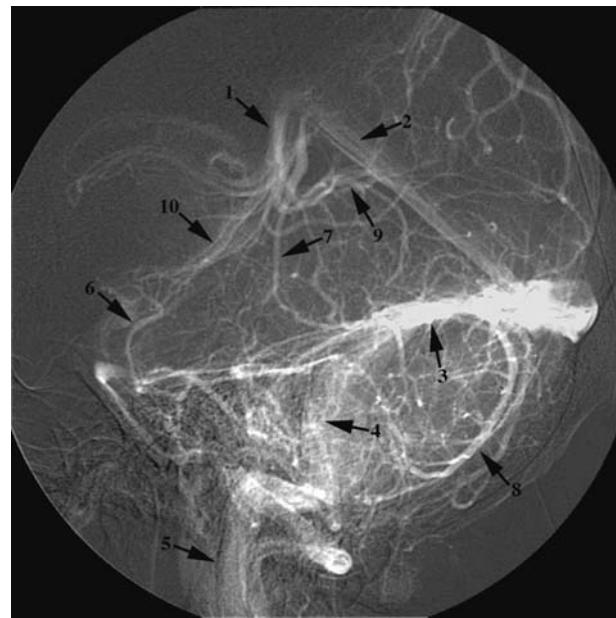


Fig. 19. Lateral protection of the venous phase of the vertebral angiogram showing the veins of the posterior cranial fossa: 1, Vein of Galen; 2, Straight Sinus; 3, Transverse Sinus; 4, Sigmoid Sinus; 5, Internal Jugular Vein; 6, Anterior Pontomesencephalic Vein; 7, Precentral Cerebellar Vein; 8, Inferior Vermian Vein; 9, Superior Cerebellar Veins; 10, Posterior Mesencephalic Vein (adjacent to the basal vein of Rosenthal).

of the brain stem (Fig. 19). The inferior vermicular veins drain into the tentorial sinus, which subsequently drains blood into either the transverse sinus or the straight sinus (Fig. 15 and Fig. 19).

5.4. Dural Venous Sinuses (Table 13)

The major dural venous sinuses are the superior sagittal sinus, transverse sinuses, straight sinus, sigmoid sinuses, and cavernous sinuses (Fig. 15, Fig. 16, Fig. 17, Fig. 18, and Fig. 19). Smaller-caliber sinuses are the inferior sagittal sinus, superior petrosal sinus, inferior petrosal sinus, and occipital sinus (Fig. 16).

The midline superior sagittal sinus and straight sinus join with the right and left transverse sinuses to form the confluence of sinuses known also as torcular herophili. The emissary veins connect the extracranial venous system to the dural venous sinuses through the cranium and the skull-base foramina.

Before reaching the right side of the heart through the superior vena cava, the intracranial venous system drains on each side into three or four major sinuses whose flow eventually goes into the internal jugular veins.

All intracranial sinuses are endothelium-lined structures bounded by thick layers of collagenous tissue derived from the dura mater. On the convexity and midline of the brain are the superior and inferior sagittal sinuses and the straight sinus, all of which converge into the torcular herophili. At the base of the skull, on each side of the pituitary fossa, is a large cavernous sinus that primarily drains veins from the orbit, the pituitary gland, anterior hypothalamic structures, and

some of the paranasal sinuses. The cavernous sinuses are located on the lateral surface of the body of the sphenoid bone. The cavernous sinus receives blood from the superior and inferior ophthalmic veins, the sphenoparietal sinus, the inferior petrosal sinus, and the basilar plexus of veins. The basilar veins receive blood from the inferior petrosal sinus, which also drains posteriorly into the marginal sinus and the anterior internal vertebral venous plexus of the spinal canal.

The cavernous sinus drains into the sigmoid sinus through the superior and inferior petrosal sinuses, two structures that run parallel to the petrous portion of the temporal bone. Both the superior and inferior petrosal sinuses communicate anteriorly and medially with the cavernous sinus. The superior petrosal sinus courses along the superior surface of the petrous temporal bone within the attached margin of the tentorium cerebelli, and the inferior petrosal sinus runs inferior and parallel to the superior petrosal sinus in the petro-occipital fissure (Fig. 15 and Fig. 16). The superior petrosal sinus drains the inferior surface of the temporal lobe and the dorsal surface of the cerebellum; a few veins from the brain stem may also reach this sinus.

The cavernous sinuses are unique venous structures that contain the cavernous segments of the internal carotid arteries and the abducens nerve (sixth cranial nerve). The oculomotor nerve (third cranial nerve), trochlear nerve (fourth cranial nerve), and maxillary and ophthalmic divisions of the trigeminal nerve (fifth cranial nerve) lie within the lateral wall of the cavernous sinuses. The cavernous sinuses are located on the lateral walls of the body of the sphenoid bone. The superior and inferior ophthalmic veins of the orbits and the sphenoparietal sinus drain into the cavernous sinus. The superior and inferior petrosal sinuses connect the cavernous sinus to the sigmoid sinus and the jugular bulb, respectively. The cavernous sinuses also freely communicate with the pterygoid venous plexus in the roof of the nasopharynx through emissary veins of the skull base and the clival venous plexus.

The major source of disease associated with intracranial veins and sinuses is the occlusion of these draining channels by thrombi or by adjacent structures, such as tumors. Intracranial venous thrombosis is causally related to infectious processes involving structures located near the cavernous sinus, such as the paranasal sinuses, orbits, teeth, skull, and scalp. Infectious processes involving any of the anatomic structures located near the cavernous sinus, such as

Table 13
Dural Venous Sinuses*

Superior sagittal sinus	
Transverse sinuses	
Straight sinus	
Sigmoid sinuses	
Cavernous sinuses	
Superior petrosal sinuses	
Inferior petrosal sinuses	
Occipital sinus	
Inferior sagittal sinus	
<i>Structures drained</i>	
<i>Straight sinus</i>	
Deep venous system	
<i>The superior sagittal sinus, transverse sinus</i>	
<i>Sigmoid sinus</i>	
The superficial cortical venous system	
<i>Cavernous sinus</i>	
Pituitary gland	
Orbits	
Inferior hypothalamus	
Part of the posterior paranasal sinuses	
<i>Superior and inferior petrosal sinuses</i>	
Brain stem	
Ventral surface of the cerebellar hemispheres	
<i>Torcular herophili</i>	
Confluence of the superior sagittal, transverse sinuses, midline straight, and occipital sinus	

*Extensive anastomotic collateral channels exist between various dural venous sinuses.

the intraorbital contents, can lead to the thrombotic occlusion of this sinus.

There are several venous channels connecting extracranial and intracranial veins, known as *emissary veins*. The flow in these and many other cranial venous structures can be easily reversed, because there are no intraluminal valves or structures to ensure unidirectional flow.

Microscopically, the walls of the brain venules are almost indistinguishable from the capillary walls. Venous walls consist of a continuous lining of endothelial cells. The endothelium is nonfenestrated, and the junction of two apposed plasma membranes is separated only by a narrow cleft, except at the luminal surface, where the membranes form zonulae occludentes. The walls of cerebral veins consist of an endothelium-lined tunica intima surrounded by an adventitial layer. Smooth-muscle cells are not a common component of the venous wall. The wall of dural sinuses consist of an inner lining of endothelium and an outer layer with essentially the same architecture as the dura mater. This outer layer consists chiefly of fibroblasts and large interlaced bundles of collagenous fibers. The arachnoid villi appear shortly after birth, and with advancing age, they form cauliflower-like clusters referred to as pacchionian granulations. They are primarily located along the walls of the superior sagittal sinus and the transverse sinus. The arachnoid villi are intimately involved in the process of transferring spinal fluid from the subarachnoid space to the dural venous sinuses.

Intracranial veins do not collapse, even at transmural pressure of 1.0 mm Hg, which contrasts with the process in extracranial veins.

Acknowledgements: This chapter is dedicated to the memory of the late Dr. Julio H. Garcia, whose leadership and enthusiasm was the source of the original chapter and this revision.

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Stroke

Gregory Cooper, Gerald Eichhorn and Robert Rodnitzky

Stroke is the third leading cause of death in the United States, with approximately 700,000 new or recurrent cases each year. The prevalence within the United States has been estimated at 4.7 million, with a cost of \$51.2 billion annually. Stroke refers to the neurologic dysfunction resulting from a derangement of the blood supply to the brain or spinal cord. The neurologic symptoms of a stroke are often of sudden onset and may be temporary or permanent. Strokes may be ischemic, resulting from impaired blood flow, or hemorrhagic. Cerebral ischemia is a potentially reversible alteration of brain function that results from inadequate delivery of critical blood-borne substrates such as oxygen and glucose. Cerebral infarction occurs if ischemia is severe enough to kill cells. In this circumstance, there is a high likelihood of permanent dysfunction. The brain is particularly sensitive to severe ischemia, with irreversible cell death resulting in 2 to 3 minutes in some species and in approximately 6 to 8 minutes in humans.

PATHOLOGIC MECHANISMS OF STROKE

Loss of Energy-Dependent Homeostatic Mechanisms Leads the Neurologic Symptoms That Result from a Stroke

With decreasing or absent blood flow to the affected brain region, anaerobic metabolism of glucose leads to accumulation of lactate and acidosis. Subsequently, energy-dependent ion homeostasis across cell membranes begins to fail, resulting in an influx of calcium, sodium, and chloride ions along with water. The enhanced cellular water content further damages individual cells and leads to regional brain swelling, which can compress neighboring blood vessels, further reducing blood supply to the injured area. As a result of

intracellular acidosis related to enhanced anaerobic glycolysis, mitochondrial respiration is further depressed, free radical formation is enhanced, and lipid peroxidation occurs. Excitotoxicity results from overstimulation of neuronal glutamate receptors. Cell structure degenerates, because energy, in the form of ATP, is required to support resynthesis of macromolecules in the normal course of cell maintenance. With increasing time, or worsening ischemia, this process becomes irreversible.

Normal Homeostatic Mechanisms in the Cerebral Vascular System May Be Lost Because of Cerebral Ischemia

The process of vascular autoregulation maintains a relatively constant cerebral blood flow despite variations in mean arterial pressure. This system is generally effective as long as the mean arterial pressure does not fall below 60 mm Hg or rise above 150 mm Hg. As a result of autoregulation, cerebral blood flow increases when there is an elevation of arterial PaCO_2 . In an area of infarcted brain, autoregulation is typically lost, and the cerebral blood flow passively mirrors changes in systemic blood pressure so that there is virtually no compensation for extremely low or extremely high pressures. The lost ability to respond to increased levels of PaCO_2 prevents a normal compensatory increase in blood flow in response to potentially damaging acidosis.

THE NORMAL BLOOD SUPPLY TO THE BRAIN CAN BE DISRUPTED BY SEVERAL MECHANISMS

Blood flow to the brain is diminished by any process that significantly narrows or occludes a nutrient blood vessel. Narrowing of a blood vessel without

total occlusion is referred to as stenosis. In a large cerebral blood vessel such as the carotid artery, a substantial stenosis of 50% to 75% is required before blood flow is seriously diminished. Even under these circumstances, cerebral blood flow can remain relatively normal if collateral circulatory pathways compensate by shunting more blood to the affected region of the brain. Narrowing or occlusion of a cerebral artery is usually the result of lipid-laden atherosclerotic deposits attached to the inner surface of the vessel.

If the atherosclerotic deposit does not itself occlude an artery, it may serve as a nidus for the formation of a superimposed occluding blood clot, called a *thrombus*. Occlusion of a critical blood vessel can occur when a blood clot from a distant site, such as the heart, travels through the arterial system and lodges in a blood vessel of smaller caliber within the brain. A clot from a distant origin is referred to as an *embolus*. Cholesterol particles that have broken loose from an area of atherosclerosis and traveled downstream in the vascular system constitute another form of embolus.

A drop in cerebral perfusion pressure can result in diminished blood flow to the brain despite fully patent vessels. Such a drop, usually related to low blood pressure, can be the cause of watershed or border-zone infarctions. The terms *watershed* and *border zone* refer to areas of the brain between the terminal distributions of two adjacent arteries, such as the anterior and middle cerebral arteries. Because they are at the end of the pipeline, such regions are subject to low, marginally adequate arterial pressure under normal circumstances. They are therefore the first to fail when blood pressure in the system drops further. If a drop in blood pressure is sufficiently severe and sustained, the entire brain is affected, resulting in *global cerebral ischemia*.

Cerebral hemorrhage, one of the most severe forms of stroke, results from the spontaneous rupture of the wall of a blood vessel that has been weakened by long-standing high blood pressure or from the rupture of a cerebral *aneurysm*, a balloon-like outpouching of the layers of an arterial wall. In the former case, bleeding usually occurs directly into the brain, resulting in an intracerebral hemorrhage. In the latter circumstance, hemorrhage may also occur within the brain substance, but because aneurysms are more typically found on the surface of the brain, hemorrhage into the CSF contained within the subarachnoid space is much more common. Intracerebral and subarachnoid hemorrhages are extremely serious, and either can be fatal, one because of mass effect and compression of adjacent structures within the brain and the other because of associated severe arterial spasm provoked by blood in the CSF.

TRANSIENT ISCHEMIC SYMPTOMS OFTEN PRECEDE CEREBRAL INFARCTION

Under certain circumstances, the symptoms of cerebral ischemia may last only several minutes and then resolve. These episodes are known as *transient ischemic attacks*. By definition, a transient ischemic attack is an episode in which symptoms persist for less than 24 hours before resolving, but most last less than 15 minutes. A common cause of a brief ischemic episode is the passage of an arterial embolus downstream, resulting in the temporary occlusion of a smaller blood vessel until the embolus breaks up and is flushed downstream. These emboli can arise from the heart or from an atherosclerotic lesion in a large blood vessel. The point at which the common carotid artery bifurcates into its internal and external branches is a common site of severe atherosclerosis and often serves as a staging ground for fibrin-platelet or cholesterol emboli. Emboli from this site can travel to the brain, producing sensory, motor, or language dysfunction, or to the arterial supply of one eye, producing the classic syndrome of amaurosis fugax (e.g., fleeting blindness) before they break up. In this condition, the affected person describes a brief episode during which it appears that a fog or a cloud has descended like a window shade over the entire visual field of one eye.

Transient ischemic attacks may also result when a region of the brain being perfused by a severely stenotic artery is temporarily subjected to abnormally low perfusion pressure during an episode of systemic hypotension. This further reduces the already marginal blood supply, resulting in transient neurologic symptoms until the blood pressure returns to normal.

Transient ischemic attacks can be repetitive and stereotypical. Because they are often the forerunner of a subsequent episode that produces a permanent neurologic deficit, a transient ischemic attack mandates immediate investigation to try to identify and correct its underlying cause and prevent a more devastating, irreversible ischemic event.

THE NEUROLOGIC DEFICIT IN ISCHEMIC STROKE DEPENDS ON WHICH BLOOD VESSEL IS INVOLVED

The brain is perfused by the paired carotid arteries and the vertebral basilar system of blood vessels. The neurologic signs and symptoms associated with a stroke depend on which of these blood vessels or their branches are involved.

One of the most common vascular territories to be involved in stroke is that of the *middle cerebral artery*. The middle cerebral artery has deep (e.g., lenticulostriate) and superficial (e.g., pial) branches. The deep branches perfuse the corona radiata, portions of the internal capsule, and parts of the globus pallidus and caudate nucleus. The pial branches provide blood supply for most of the lateral surface of the frontal, temporal, and parietal lobes. The clinical syndrome resulting from middle cerebral artery stenosis or occlusion depends on which of its branches are most involved. Among the most common clinical findings associated with middle cerebral artery involvement are contralateral limb paralysis and contralateral sensory loss, both involving the arm much more than the leg. The relative sparing of the lower extremity reflects the fact that its representation in the primary motor and sensory cortex is on the medial surface of the frontal and parietal lobes, respectively, areas that are outside the middle cerebral artery perfusion zone. The eyes may become deviated toward the side of the lesion because of destruction of the frontal lobe gaze center responsible for directing rapid eye movements in the horizontal plane to the contralateral side. When this center is damaged, only the intact gaze center in the opposite hemisphere continues to drive gaze, and the eyes are involuntarily directed toward the side of the frontal lobe infarction. Because the speech area is within the middle cerebral artery territory, aphasia is common in dominant hemisphere infarctions. In nondominant hemisphere infarction, especially those involving the parietal lobe, there may be severe disturbances of spatial function, such as *hemispatial neglect*, which is a tendency not to attend to objects or stimuli located in space on the side opposite the infarction. Separate and distinct from hemispatial neglect, there can be contralateral *hemianopia*, which is a loss of half the visual field caused by the involvement of the optic radiations coursing through the temporal and parietal lobes.

The *anterior cerebral artery* perfuses the anterior frontal lobe and the parts of the frontal and parietal lobe on the medial surface of the hemisphere. Because the lower-extremity portions of the motor and sensory homunculus are located on the medial hemispheric surface, anterior cerebral artery infarction preferentially produces paralysis and sensory loss in the lower extremity. Aphasia or visual field loss is not typically part of this syndrome.

Occlusion of the *internal carotid artery* can result in infarction of the entire anterior two-thirds of the hemisphere, which constitutes the perfusion area of

its two major branches, the anterior cerebral and middle cerebral arteries. Because the anterior cerebral artery often receives significant collateral flow from the opposite anterior cerebral artery through a vessel connecting the two, an internal carotid artery occlusion often results in damage confined largely to areas perfused by the middle cerebral artery.

Occlusion of one *vertebral artery* may go unnoticed if the opposite vertebral artery is patent and allows adequate blood flow into the basilar artery. However, vertebral artery occlusion can result in an infarction of the structures perfused by one of its branches, the *posterior inferior cerebellar artery*. Occlusion of this artery results in infarction of the lateral medulla, and the resultant constellation of neurologic signs, known as *Wallenberg's syndrome*, is one of the most striking examples of predictable clinico-anatomic correlation in clinical neurology. Structures that are affected in a lateral medullary infarction include the spinal tract of the trigeminal nerve, the spinothalamic tract, the nucleus ambiguus, the inferior cerebellar peduncle, and the sympathetic fibers descending through the brain stem from the hypothalamus.

The neurologic deficit occurring in Wallenberg's syndrome coincides exactly with the function of these structures. Sensory symptoms consist of loss of pain and temperature perception but not touch perception on the ipsilateral side of the face (e.g., uncrossed spinal tract of trigeminal nerve) and loss of pain and temperature sensation on the contralateral side of the body (e.g., crossed spinothalamic tract). There is ipsilateral limb incoordination (e.g., cerebellar peduncle) and a raspy, breathy voice caused by paralysis of the ipsilateral vocal cord (e.g., nucleus ambiguus). In the ipsilateral eye, a small pupil and a drooping eyelid (e.g., Horner's syndrome) are caused by interruption of the descending sympathetic fibers.

Wallenberg's syndrome also illustrates an important principle of clinical neuroanatomic localization. Crossed motor or sensory symptoms, such as involvement of one side of the face and the other side of the body, usually imply brain-stem pathology when caused by brain infarction.

Occlusion of the *basilar artery* can result in infarction of the entire upper brain stem and both occipital lobes. The resultant massive brain-stem dysfunction is often fatal. The paired posterior cerebral arteries originate from the bifurcation of the terminal portion of the basilar artery. Each posterior cerebral artery has a hemispheric branch that supplies the occipital cortex and penetrating branches that perfuse the

midbrain in concert with similar branches from the basilar artery. Occlusion of the hemispheric branches on one side causes loss of the opposite half of the visual field (e.g., hemianopia), which is identical (e.g., homonymous) in both eyes. An occlusion of the right posterior cerebral artery resulting in a right occipital infarction causes loss of the left half of the visual field from the right and left eyes.

Occlusion of the hemispheric branches to both occipital cortices results in a form of total visual loss known as *cortical blindness*. Cortically blind persons often deny their visual disability. Each posterior cerebral artery also perfuses the splenium of the corpus callosum. When this structure is infarcted along with the primary visual cortex of the dominant hemisphere, the syndrome of *alexia without agraphia* results. This can be viewed as a disconnection syndrome in which the visual input from the visual cortices are disconnected from the perisylvian cortices, in essence preventing a decoding of the written information. The syndrome of *hemachromatopsia*, which is an inability to perceive color, occurs when the inferior, medial occipital lobe is infarcted. The cells in this area of the visual cortex are wavelength-selective in response to light and form the basis of color recognition. As is the case with hemianopia, the visual field opposite the side of the lesion is affected.

LACUNAR INFARCTIONS DO NOT CONFORM TO THE DISTRIBUTION OF MAJOR CEREBRAL ARTERIES

Lacunar infarctions are small lesions, usually less than 15 mm in diameter. They are thought to result from occlusion of small, penetrating arteries that have been damaged by chronically elevated arterial blood pressure. Although very small, lacunar infarctions may occur in strategic areas, such as the internal capsule or the pyramidal tract in the pons, where they can cause severe hemiparesis. A lacunar infarction involving the posterior ventral nucleus of the thalamus can cause isolated, severe contralateral sensory loss.

TREATMENT OF STROKE

The treatment of stroke can be divided into the initial and subsequent management of the acute stroke itself, and secondary prevention of further strokes. In ischemic stroke, in addition to basic supportive measures, acute treatment is based on the restoration of normal blood flow. This may be accomplished through

the use of thrombolytic agents, such as the tissue plasminogen activator (tPA) given either intravenously or intra-arterially at the site of thrombus. Mechanical recanalization may also be attempted at the time of angiography. A device has recently been developed that retrieves the blood clot causing ischemia at the time of angiography, allowing the possibility of early recanalization and restoration of blood flow to the affected artery and brain tissue.

Patients with hemorrhagic strokes are often admitted to an intensive care unit and treated supportively. Surgical removal of blood clots that are of sufficient mass to dangerously compress adjacent vital brain structures is sometimes required. A ruptured aneurysm is treated by occluding or tying off the weakened arterial bleed so that it cannot bleed again. Medical therapy is directed at preventing blood vessels from going into spasm in response to the presence of blood in the subarachnoid space. Certain calcium channel-blocking agents are useful for this purpose.

Early institution of intensive rehabilitation programs after the stroke may improve functional outcome. It has also been shown that admission to an inpatient rehabilitation unit after stroke leads to a better outcome.

Secondary prevention of stroke is, in part, guided by the mechanism of stroke. In ischemic stroke, strategies include prevention of blood-clot formation and improvement of blood-vessel patency. For persons who have experienced an episode of cerebral ischemia, agents such as aspirin, which inhibit platelet aggregation, are of major benefit in preventing recurrent ischemic events. More potent anticoagulant drugs such as warfarin can be used to prevent larger clots in the heart or blood vessels from forming or, once formed, from breaking loose and entering the cerebral circulation. A patient who has had a transient ischemic event or complete stroke as a result of severe carotid artery stenosis often benefits from surgical removal of the atherosclerotic deposit responsible for the arterial narrowing. Experience suggests that stroke patients who have arteries with the highest degree of blockage, in excess of 70%, benefit the most from surgical treatment, with a definite reduction in the risk for future ischemic events. Accessibility considerations limit such surgical procedures largely to the common carotid artery and the extracranial portion of the internal carotid artery, the most common site of surgery. More recently, angioplasty and stenting of stenosed arteries has been employed.

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Choroid Plexus–Cerebrospinal Fluid Circulatory Dynamics: Impact on Brain Growth, Metabolism, and Repair

Conrad E. Johanson

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1. STRUCTURAL AND FUNCTIONAL COMPONENTS OF THE CEREBROSPINAL FLUID

Cerebrospinal fluid (CSF) has a major impact on the fluid environment of neurons. Choroid plexus (CP) tissue in the four ventricles generates 70% to 80% of actively secreted CSF that derives from the carotid and vertebral systems. Upon flowing from choroidal origins to distal sites, CSF contacts other membranes that encompass the hemispheres: the ependyma of the ventricles and the pia/arachnoid of the subarachnoid space (SAS). Consequently, the composition of flowing CSF and adjacent brain

interstitium is progressively modified by bidirectional exchanges of water, ions, and proteins (1, 2).

The CSF, then, is a dynamic system working in parallel with cerebral capillary transporters to optimize the neuronal environment. Disrupted transport at the blood-CSF interface (choroid plexuses mainly) and blood-brain barrier (BBB) compromises cerebral function. Although long known that stable brain fluid volume and composition vitally depend on CP function, it is now more evident that the streaming CSF dynamically interacts with the brain. Accordingly, less efficient exchange of solutes between CSF and the brain, as in aging and disease (3), impairs neuronal activity.

As part of the proximal CSF system, CP is viewed as a “port of entry” for many substances fluxing into the central nervous system (CNS). The unique array of transporters in the choroid epithelial basolateral

(plasma-facing) membrane, compared with the counterpart luminal membrane of brain endothelium, affords opportunities for selectively translocating agents into the CSF-brain domain (4). That is, upon transport from blood to CSF, a substance readily accesses neurons because little resistance to diffusion is offered by permeable gap junctions between CSF-bordering cells. Consequently, the CP-CSF nexus is an “industrious gateway” for supplying numerous endogenous and exogenous agents to the CNS (5, 6).

2. DIVERSE ROLES OF CSF IN EFFECTING BRAIN WELL-BEING

As an active secretion into the ventricles by CP epithelium, the CSF helps to establish a specialized extracellular environment for neurons. Anatomically, the relationship of CP-CSF to brain and spinal cord is depicted in Fig. 1. Suspended in the ventricles, the CPs generate an avascular, nonlymphatic fluid that acts like a “third circulation.” Continual formation and drainage of CSF allows this unique circulatory system to perform diverse metabolic and signaling functions (7). Several physical and biochemical attributes of CSF are summarized in Table 1.

2.1. Buoyancy Effect of Suspension Fluid

CSF is 99% water. The buoyancy of CSF protects the brain against the shearing forces of acceleration and deceleration. The relative specific gravity of CSF (~1.007) versus that of nervous tissue (~1.040) enables the 1400 g human brain to weigh about 45 g when suspended in CSF. Consequently, this >30-fold reduction in effective weight minimizes injury by reducing brain momentum in response to stresses/strains inflicted on the head. Angular acceleration, as in severe trauma, may override the normally buoyant effect of CSF. This can tear or herniate cerebral tissues.

2.2. Intracranial Volume Adjustor

By compensatory mechanisms, CSF volume is increased or decreased to stabilize intracranial pressure (ICP). Rapidly reduced CSF volume, resulting from enhanced CSF absorption, is the response to elevated ICP. On the other hand when ICP falls, the CSF volume increases by the slowing of absorption. The ability of CSF volume to adjust freely to altered ICP is the basis for the Monro-Kellie doctrine. This long-established physiologic principle recognizes that the brain, together with CSF and blood, are encased in a rigid chamber. Because tissue and fluid contents

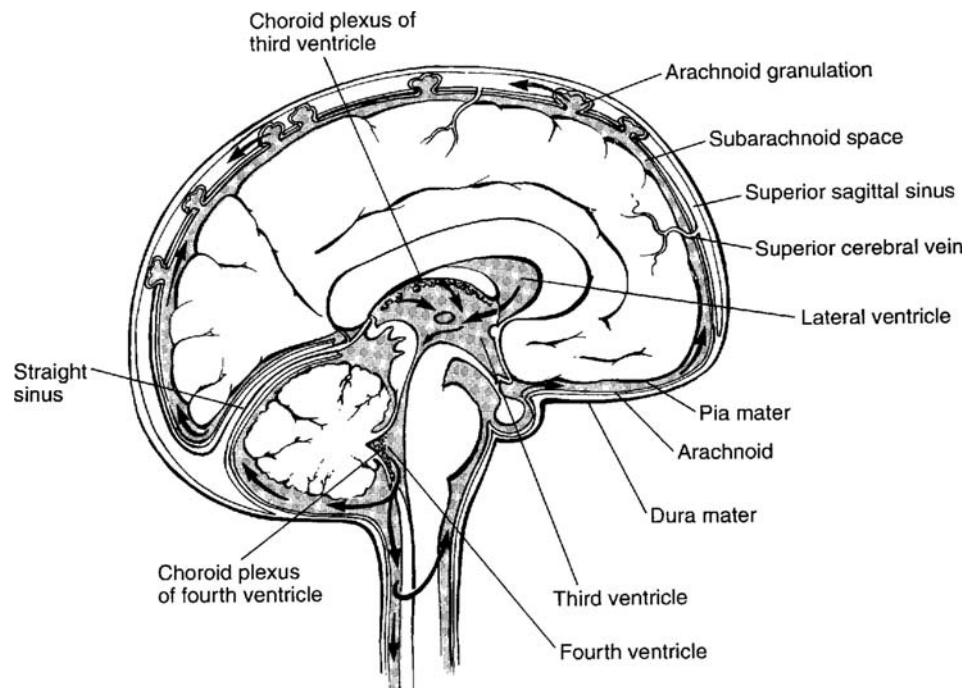


Fig. 1. CSF is formed and secreted by choroid plexus (CP) in the lateral, third, and fourth ventricles. The great vascularity of the plexus imparts a reddish cast to these tissues. In adult humans, the total weight of CPs in the four ventricles is about 2 to 3 g. Choroidal tissue is not present in SAS surrounding the brain hemispheres and spinal cord.

Table 1
Roles of CSF in Serving the Brain

<i>CSF functions</i>	<i>Examples</i>
Buoyancy effect	Because the brain weight is effectively reduced by more than 95%, shearing and tearing forces on neural tissue are greatly minimized.
Intracranial volume adjustment	CSF volume can be adjusted, increasing or decreasing acutely in response to blood volume changes or chronically in response to tissue atrophy or tumor growth.
Micronutrient transport	Nucleosides, pyrimidines, vitamin C, and other nutrients are transported by the choroid plexus to CSF and eventually to brain cells.
Protein and peptide supply	Macromolecules like transthyretin, insulin-like growth factor, and thyroxine are transported by the choroid plexus into CSF for carriage to target cells in the brain.
Source of osmolytes for brain volume regulation	In acute hypernatremia, there is bulk flow of CSF with osmolytes, from ventricles to surrounding tissue. This promotes water retention by shrunken brain, i.e., to restore volume.
Buffer reservoir	When brain interstitial fluid concentration of H^+ , K^+ , and glucose are altered, the ventricular fluid can help to buffer the extracellular fluid changes.
Sink or drainage action	Anion metabolites or neurotransmitters, protein products of catabolism or tissue breakdown, and xenobiotic substances are cleaned from the CNS by active transporters in the choroid plexus or by bulk CSF drainage pathways to venous blood and the lymphatics.
Immune system mediation	Cells adjacent to ventricles have antigen-presenting capabilities. Some CSF protein drains into cervical lymphatics, with the potential for inducing antibody reactions.
Information transfer	Neurotransmitter agents like amino acids and peptides may be transported by CSF over distances to bind receptors in the parasympathetic mode.
Drug delivery	Some drugs do not readily cross the blood-brain barrier but can be transported into the CSF by endogenous proteins in the choroid plexus epithelial membranes.

are practically incompressible, a change in volume of any constituent must be balanced by an equal and opposite effect in another component(s). Except for pathologic changes in brain bulk, the most common displacements of CSF occur in response to acutely altered blood volume (e.g., secondary to CO_2 -induced vasodilation or constriction of the cerebrovascular bed).

2.3. Micronutrient Supply System for Neuronal Networks

Secretory mechanisms in choroid epithelial cells transport many water-soluble substances (e.g., micronutrients) into CSF for eventual carriage to brain target cells. These substances are needed in nano- to micromolar concentrations over extended periods. Micronutrients transported into CSF include vitamin C, folates (4), deoxyribonucleosides (8), vitamin B₆ (9), and certain trace elements. Active transport pump-like carriers in the CP epithelium pull these micronutrients across the blood-facing membrane of the plexus. Such substances are subsequently

transported from choroidal cytoplasm to CSF by facilitated diffusion in the apical membrane. Thereafter by bulk flow and passive diffusion, these micronutrients are widely distributed across the ventricular ependyma, and more distally in the subarachnoid system across the pia/glial lining. Ascorbate, or vitamin C, is a prototype micronutrient actively secreted across CP but not cerebral capillaries (4). Accordingly, the CP-CSF nexus is the major “gateway” for nourishing the brain.

2.4. Distributor of Centrally Synthesized Peptides, Growth Factors, and Proteins

CSF is a dynamic distribution pathway for communication and integration of peptide signals *within* the CNS. CP is important in the CSF conveyance and/or reception of such peptidergic “signals.” Membrane receptors in CP for arginine vasopressin (AVP), atrial natriuretic peptide (ANP), and angiotensin II indicate that centrally released peptides secreted into CSF modulate choroidal blood flow and secretion (10–12). CP synthesizes many growth

factors (13), including insulin-like growth factor II (IGF-II). After secretion into CSF, IGF-II accesses the parenchyma to exert metabolic and trophic effects. Transthyretin (TTR), the main protein secreted by CP, mediates thyroid hormone T₄ transport from blood to CP to CSF (14). Synthesized early in ontogeny and phylogeny, TTR has multiple effects in higher vertebrates. In aging, less TTR is synthesized by CP (15). This deficiency leads to beta-amyloid fibrils and plaque formation in brain (16). Thus CP-CSF convects an array of macromolecules essential to brain development, metabolism and health.

2.5. Source of Osmolytes for Brain and Cord

CSF has a relatively high concentration of NaCl (i.e., 15% to 20% greater than serum). Under certain conditions, the Na and Cl in CSF serve as inorganic osmolytes to restore brain volume decreased by water loss to blood. In acute hypernatremia, the brain shrinks because there is net water movement from CNS to hypertonic plasma. This initial compensation of CSF movement (with its inorganic ions) from ventricles to brain (17) precedes the osmotic adjustment several days later of a parenchymal increase in organic osmolytes such as inositol and taurine.

2.6. Buffering Reservoir: Biochemical and Biophysical

Chemical and physical buffering occurs in CSF. Minimally incremented ion concentrations in brain interstitial fluid (ISF) result from ventricular buffering of ions received from the interstices. Seizures and ischemia, respectively, promote ISF retention of K and acids. Ion buildup in ISF promotes diffusion down concentration gradients into CSF. Thus, the large-cavity CSF reservoir is a “buffer medium” to accommodate brain “spillovers.” By volume dilution, and active transport by plexus to remove K from CSF or neutralize H by secretion of HCO₃ (18), there is minimal fluctuation of extracellular ions. CSF [K] is buffered by the Na-K pump and NaK2Cl transporters (19) in the apical membrane of CP epithelium.

Physical buffering also occurs in CSF (i.e., the dampening of vascular pulsations transmitted to the ventricles by brisk choroidal hemodynamics). Functioning like a “shock absorber,” the CSF attenuates its own pulsatile motion that if, not appropriately buffered, injures the delicate nervous tissue contiguous with the ventriculo-subarachnoid spaces. Thus, CP vascular pulsations can dilate the ventricles upon increased impedance to the flow of CSF pulsations

through the SAS. Such ventriculomegaly is the outcome of redistributed CSF pulses from the subarachnoid compartment back to the ventricles and the capillary-venous circulation (20). Some forms of communicating hydrocephalus may thereby result from pulse redistribution within the cranial cavity. Accordingly, the physical aspect of CSF dynamics, including impedance to flow, importantly determines the configuration and volume of large-cavity CSF.

2.7. Excretor of Catabolites, Proteins, and Toxins

In addition to supplying substances for brain anabolism and maintenance, the CSF also removes and excretes various catabolites from neurons and glia. There is a drain from brain ISF into CSF of the organic anions 5-OH indoleacetic acid (5-HIAA) and homovanillic acid (HVA) (i.e., metabolites of serotonin and dopamine). Once in CSF, these organic anions are actively reabsorbed by CP into blood or cleared convectively by bulk flow of CSF into venous blood. Such removal or “sink action” is exerted on numerous organic anions and cations as well as proteins and peptides. Iodide ion, especially toxic to brain, is avidly transported from CSF by CP.

Some antibiotics and other useful agents are efficiently cleared from CSF by CP, thereby reducing CSF concentrations to subtherapeutic levels. Organic solute transporters (21), such as P-glycoprotein (Pgp or MDR1), the low-density lipophilic receptor related protein (LRP-1), and the multidrug-resistant protein 1 (MRP1) in the plasma membranes of CP (and endothelium of cerebral capillaries), actively transport many drugs out of the CNS. Pharmacologic manipulation of the Pgp, LRP-1, and MRP transporters is a significant challenge to pharmaceutical drug designing.

2.8. Mediator of Immune Responses Within the CNS

The CP-CSF system mediates immunologic communication between brain and periphery. The plexus epithelium presents antigen to, and stimulates proliferation of, peripheral helper T lymphocytes. Moreover, CSF proteins drain by bulk flow along the SAS that envelops optic and olfactory nerves. Because such drainage eventually passes through cervical lymph, there is a potential for CSF antigenic material to elicit nodal antibody reactions. Such immunologic responses to proteins draining from CSF affect interactions between central and immune systems in

diseases (e.g., multiple sclerosis or allergic encephalitis) in which certain CSF proteins display antigenicity. Leukocyte trafficking across CP is problematic in controlling brain autoimmune diseases (22).

2.9. Neuroendocrine Conduit for Neurotransmitter and Hormonal Signals

Transmitters are moved by CSF over considerable distances to bind receptors in the parasympathetic mode. Neurotransmitters escaping the microenvironment of local synapses can be distributed by bulk flow between ISF and ventricular cavities. Arterial pulsations propel the extracellular fluid containing “informational molecules” along perivascular and subependymal pathways. Mismatches between receptors and ligands, seen by light microscopy, imply parasympathetic transmission. Gamma-aminobutyric acid exemplifies a “mismatched” transmitter acting at a distance between brain and ventricles. In this manner, CSF bulk flow along circumscribed routes mediates parasympathetic transmission. Such CSF convection is known as volume transmission (1).

Peripherally derived hormones are also “ferried” along CSF pathways to integrate signaling between distant regions. Prolactin in plasma uses the CP-CSF nexus to carry its “hormone signal” to central targets. Receptors in CP are regulated by plasma prolactin, which changes in pregnancy and lactation (23). Receptor-mediated transport of prolactin from blood to CSF conveys specific neuroendocrine information by bulk flow to the third ventricle–hypothalamus region where ependymal tanycytes likely transport prolactin to the hypothalamic-hypophysial axis. Consequently, there is the appropriate increase or decrease in synthesis/release of prolactin into plasma for feedback regulation of prolactin-sensitive cells in reproductive organs. In this way, CP serves as a “relay station” to coordinate prolactin transfer between peripheral and central neuroendocrine regions.

2.10. Alternate Drug Delivery Route to Circumvent the Blood-Brain Barrier

In treating brain cancer and other neural disorders, it is difficult to get water-soluble drugs to CNS targets. A novel approach promotes drug passage across the blood-CSF interface by using therapeutic agents transportable by CP endogenous protein carriers (6). An example is the anti-AIDS agent

azidothymidine (AZT), with affinity for the nucleoside transporter in CP (24). Accessing the CSF, AZT then penetrates the ependyma interfacing the ventricular CSF with brain ISF. Agents transported within the CP-CSF-ependymal nexus (24, 25) reach tumors or stem cells (8) along ventricular borders. Combinatorial strategies involving bacteriophage can identify novel peptides with affinity for CP epithelium. This holds promise for manipulating the blood-CSF gateway (6, 24, 25).

3. PIVOTAL MODULATORY FUNCTIONS OF THE CSF IN FETAL BRAIN DEVELOPMENT

Embryologically, the ventricular system begins when neural groove closure forms a tube. The earliest fluid in the neural tube precedes CP appearance and so is not true CSF. Ciliary action within fetal ventricles mixes the fluid and promotes diffusional exchange across the tube wall. Early fetal brain fluid is retained in the ventricles because it cannot escape into the SAS.

The major ventricular components appear at early stages (Fig. 2, top). Lateral ventricles are spherical and close to the midline at 2 months. During the second trimester, a portion of the lateral first and second ventricles expands laterally as cerebral hemispheres enlarge. Posterior and inferior expansion of the brain forces the cortex into a “C” shape. Consequently, the underlying lateral ventricles, caudate nucleus, and hippocampal formation are also molded into a “C” shape. At birth, the shape of the ventricular system resembles that in adulthood (Fig. 2, right).

CP tissue first appears in human ventricles during the second month of intrauterine life. There are several stages of choroidal differentiation. By the third gestational month, the plexuses nearly fill the lateral ventricles. Thus fetal CP, relative to brain, is proportionately larger than in adults and fills more ventricular space. This intimates that the CP-CSF ventricular fluid prominently provides nutrients to fetal neural tissue with its low capillary density and blood flow.

Germinative matrix in the ependymal wall supplies progenitor cells for fetal brain. Stem cells give rise to neurons in the cell layer under the ependyma. FGF-2 and TGF- β promote division and differentiation of these primitive cells. Growth factors are supplied by the synthesizing CP and ependyma to the subventricular zone (SVZ). Regulated transport of growth

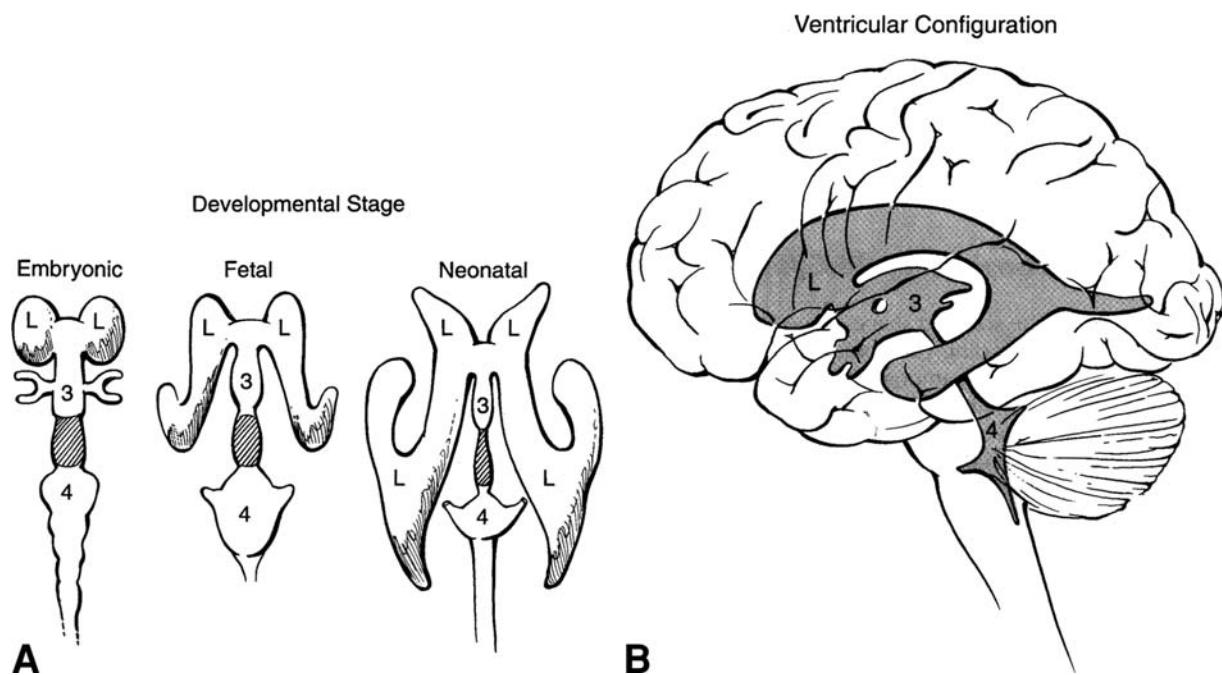


Fig. 2. (A) Shape of the ventricular system at early stages of development. Even by the second month of the first trimester, all of the major components of the ventricles are present. In the 5-month fetus, the first and second ventricles grow laterally as cerebral hemispheres enlarge. By birth, the general configuration of the ventricular system is similar to that of adults. (B) Physical configuration of the cerebroventricular system of mammalian brain: Right and left lateral ventricles are located in the medial portion of their respective hemispheres. The third ventricle, which is smaller and in the midline, is physically contiguous with the anterior horns of the lateral ventricles above and the fourth ventricle below. The shapes of the four ventricles are discussed in the text, and the interventricular foramina/channels are summarized in Table 2.

factors, hormones, and other proteins from CSF to SVZ importantly modulates stem cell conversion to neurons (8).

4. PHYSICAL DIMENSIONS OF THE ADULT CSF SYSTEM

The size and shape of the CSF system affects the kinetics of drug distribution among CNS regions, the neuroendocrine integration of fluid balance, and extracellular aspects of neurotransmitter/peptide signaling. Interior or proximal cerebroventricular CSF generated by CP percolates down the ventricles to the

cisterns. The attenuation of the ventricular system into the narrow sylvian aqueduct gives rise to flow vulnerability. The more exterior or distal SAS lacks tufts of CP but has villi/granulations/pore-like structures to facilitate fluid outflow. SAS is therefore involved more with reabsorption than secretion.

Ventricles are linked by channels or foramina. Ventricular CSF flows from telencephalon to rhombencephalon, finally mixing with subarachnoid fluid at the brain's base. There the CSF flows out of fourth ventricle foramina into cisterns. The cisterna magna results from an arachnoid membrane bridge between cerebellar hemispheres and medulla. Table 2

Table 2
Channels or Narrow Ducts in the CSF

Name of channel	Location and significance
Foramina of Monro	Connect each lateral ventricle to the third ventricle; tissue adhesions may block channels.
Cerebral (sylvian) aqueduct	Connects the third ventricle with the fourth ventricle; narrowest passageway in ventricular CSF flow route and therefore the most likely site of obstruction leading to hydrocephalus.
Foramina of Luschka	Two exits located in the lateral recesses of the fourth ventricle permit access to basal cisterns.
Median foramen of Magendie	Midline at the caudal end of the fourth ventricle; direct access to the cisterna magna.

summarizes channels and pathways that connect the large cavities of CSF.

4.1. Configuration

4.1.1. VENTRICULAR CAVITIES

In higher vertebrates, the cerebroventricular system has four interconnecting cavities (Fig. 2, right), each containing CP. The two lateral ventricles, more or less symmetrical with each other, are the most prominent in size. CP lies as a narrow band of tissue on the floor of each lateral ventricle. A thin layer, the septum pellucidum, separates the lateral ventricles in the lower medial portion of the hemispheres. Thus the lateral ventricles are not physically contiguous but communicate with the third ventricle via the interventricular foramina of Monro.

Each lateral ventricle has a main body and three horn-shaped recesses. The most rostral lateral ventricle is the *anterior horn*. It angles downward into the frontal lobe and curves around the anterior portion of caudate nucleus. The *inferior horn* bends around the posterior thalamus, extends backward and then laterally downward in the temporal lobe. The *posterior horn* runs laterally and juts backward into the occipital lobe. At the *trigone*, the body divides into inferior and posterior horns.

The third ventricle, lying beneath the lateral ventricle bodies, houses the smallest choroid tissue. A thin cleft in the midline, it is located between two thalami. The third ventricle receives CSF from lateral ventricles and then passes the fluid into the sylvian aqueduct. Anatomically, the irregularly shaped third ventricle has four prolongations or recesses (Fig. 2, right). The front, lower part of this ventricle has adjacent *optic* and *infundibular* recesses. The back, upper part of the third ventricle has recesses named *pineal* and *supra-pineal* because of proximity to pineal gland.

The fourth ventricle occupies the most caudal part of the cerebroventricles. Lying well below the lateral and third ventricles, it is bounded by pons, medulla oblongata, and cerebellum. The rhombus-shaped fourth ventricle has a roof and floor. The roof is V-shaped with thin laminae of white matter between the cerebellar peduncles. A median opening at the caudal end of the roof, the foramen of Magendie, is significant hydrodynamically because CSF flows through this aperture into SAS. Part of the fourth ventricle roof is occupied by CP. The fourth ventricular plexus is T-shaped, with the vertical portion in the midline.

The floor of the fourth ventricle is divided into symmetrical halves by the *median sulcus*. Running perpendicular to this sulcus are delicate strands of transverse fibers, the *striae medullares* of the fourth ventricle. Other neuroanatomic features of the floor are the *medial eminence* and the *sulcus limitans*. The *medial eminence* is a longitudinal elevation that flanks both sides of the median sulcus. The *sulcus limitans* lies lateral to this *eminence*.

In the spinal cord, the analogue to the ventricular system is the central canal. This canal ends within the *filum terminale*. Imaging of normal human adult flow patterns reveals that CSF in the fourth ventricle is not normally contiguous with the central canal.

4.1.2. SUBARACHNOID SPACE

The SAS lies between the arachnoid membrane externally and the *pia mater* internally. In adults the SAS provides a route for CSF flow to absorptive sites of exit. The SAS covers the convexities of the cerebral hemispheres and forms a circumferential sleeve around the spinal cord (Fig. 1). Figure 3 displays representative architecture of the SAS.

Because pia intimately hugs the external contour of nervous tissue, whereas the arachnoid membrane bridges the sulci of brain and cord, relatively large pockets of SAS exist. Spaces at the brain base where the bridged-over gaps are large are called cisterns. One of the largest is the *cisterna magna*, situated between the inferior surface of the cerebellum and medulla. Because of accessibility at the foramen magnum, the cisterna magna is used to conveniently sample animal CSF. *Cisterna ambiens* is a CSF pocket dorsal to midbrain. Lying between the base of the brain and the floor of the cranial cavity are the pontine, chiasmatic, and interpeduncular cisternae.

4.2. Volume of CSF Compartments

Total CSF volume in normal adult humans is about 140 mL. Ventricular system volume is estimated by casting techniques, CAT scans, and radioisotope distribution. By averaging data from several techniques, the mean volume of the ventricular system is close to 30 mL. Thus, the composite volume of the four ventricles is about 2% of the brain volume. Studies find no correlation between ventricular volume (the range of which is 10 to 60 mL) and brain volume.

Most of the total CSF volume of 140 mL is composed of the 110 mL in the SAS of the brain and

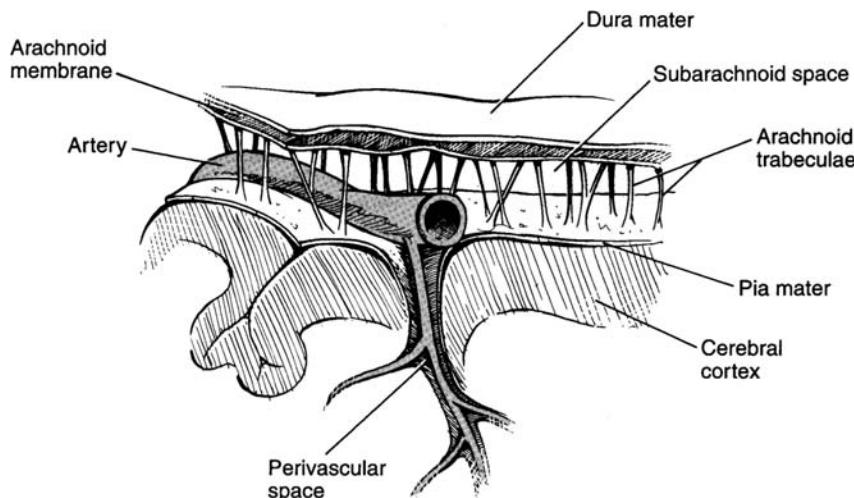


Fig. 3. Meningeal aspects of subarachnoid space: The roof of the SAS is the arachnoid membrane, whereas the floor is the *intima pia* or pia-glia (external limiting membrane of CNS). The ectodermal arachnoid and pia-glia are bridged to each other by arachnoid trabeculae. CSF percolates through SAS. Blood vessels entering and leaving nervous tissue carry arachnoid and pia-glial cuffs. Known as the Virchow-Robin space, these cuffs enable fluid movement between extracellular space and SAS.

spinal cord. CSF surrounding the spinal cord is at least 30 mL. Thus, the largest compartment of CSF is the nearly 80 mL in the SAS and cisterns enveloping the cerebrum, cerebellum, and cord.

CSF is only one of the CNS extracellular fluids (Table 3). Another major type is the ISF that bathes neurons and glia. In practice, CSF and ISF have

similar concentrations of many substances. Figure 4 gives the volume relationship between CSF and ISF. An adult brain weighing 1400 g has about 280 mL (or g) of ISF and 140 mL of CSF, for a total of 420 mL of extracellular fluid. This compares with the approximately 800 mL of fluid in the total intracellular compartment of CNS.

Table 3
Extracellular Fluids in the CNS

Fluid	Location and characteristics
ECF	Two main types: CSF in the ventricles and SAS, and the ISF that intimately bathes the parenchymal cells of brain (e.g., neurons, glial cells).
Nascent CSF	Secreted across the apical membrane of the choroid plexuses (i.e., lateral, third, and fourth ventricles) into the ventricular space, referred to as nascent or newly formed CSF; active secretion.
Ventricular CSF	Contained in the four cerebral ventricles and aqueduct; consists mainly of nascent CSF with some exchange of content with the ependymal lining and underlying brain tissue as CSF flows down the ventricular axis.
Subarachnoid CSF	Cranial or spinal SAS; mixture of ventricular fluid that has flowed into the SAS and brain fluid that has gained access to the subarachnoid spaces; subarachnoid and ventricular CSF regarded as large-cavity CSF.
Brain ISF	Actively secreted by endothelial cells in walls of capillaries in brain and spinal cord (e.g., blood-brain barrier), modified by the water and solutes that are exchanged with brain neurons and glia; undergoes transependymal exchange with ventricular fluid; chemical composition similar to that of CSF.
Cerebral endothelial secretion	Endothelium in brain capillaries, in conjunction with astrocyte foot processes on the vascular wall, actively secrete ions and solutes from plasma into the interstices of the brain; fluid-secretory capacity of the blood-brain barrier is less than of the choroid plexuses.

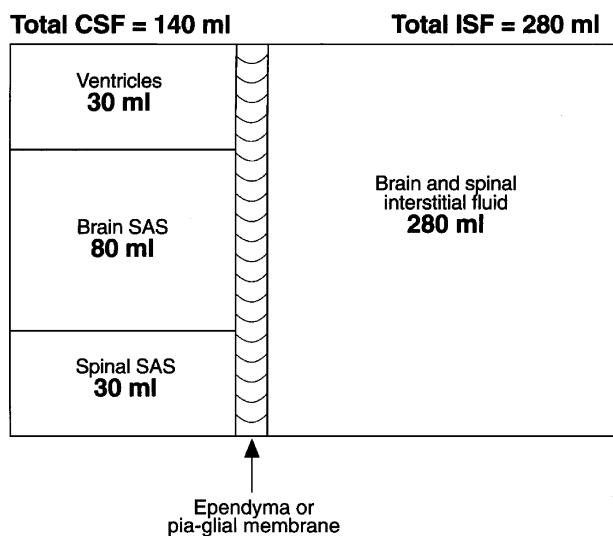


Fig. 4. An analysis of the respective volumes of fluid in CSF and interstitial space of adult humans: Nearly a third of the CNS fluid lies outside cells. A typical 1400 g adult brain contains 140 mL of CSF (10% of its weight) and approximately 280 mL of ISF that bathes neurons and glia. CSF and ISF freely communicate with each other across the permeable interfaces separating them (i.e., the ependyma in the ventricles and pia-gliar membrane in SAS).

5. CSF-BORDERING CELLS THAT DEMARCAT THE VENTRICULO-SUBARACHNOID SYSTEM

CSF is contained within and surrounds the brain and cord. CSF bathes the inside and outside surfaces of brain and is separated from the latter by a single-cell layer. In the CNS interior, a thin ependymal lining separates ventricular CSF from underlying nervous tissue. On the exterior, the pia-gliar membrane interfaces the subarachnoid CSF with adjacent cortical tissue. A third membranous interface is the choroidal epithelium, a single layer of frond-shaped epithelium that separates ventricular CSF from blood coursing through the vascular plexus. The epithelial parenchyma of CP has an ultrastructure distinct from the ependyma and pia-gliar cells (Fig. 5).

5.1. Choroid Plexus Epithelial Cell Polarity: Apical Versus Basolateral Membranes

The epithelium of CP in all four ventricles consists of tightly packed cuboidal cells with finely granular cytoplasm. A distinctive feature of CP epithelium is the *zonula occludens*, or tight junction, at apical regions between adjacent cells. This tight junction occludes the blood-to-CSF passage of hydrophilic molecules and ions. Electrical resistance associated with CP is 100-fold less than that associated with the BBB, renal distal tubule, or urinary bladder.

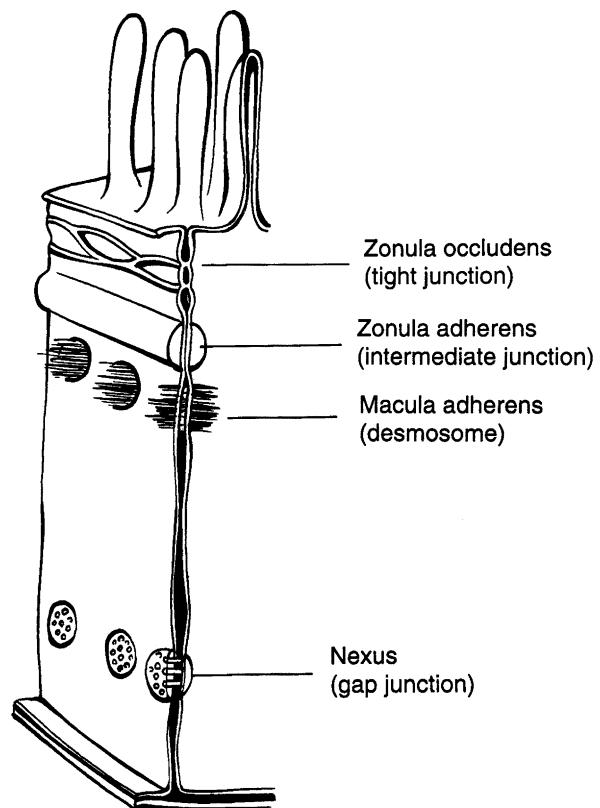


Fig. 5. Ultrastructure of intercellular junctions in CP and ependyma: An integral part of the blood-CSF barrier is *zonulae occludentes*. These tight junctions are near the apical (CSF-facing) borders of CP epithelium where cells abut. Tight junctions are multilayered membranes that completely envelop cells, thus restricting the diffusion of many solutes between plasma and CSF. On the other hand, gap junctions are between cells of the ependymal and pia-gliar linings. Gap junctions form incomplete belts around cells, therefore these intercellular junctions are more “leaky” than are tight junction counterparts in CP. Overall, CSF composition resembles brain ISF more than plasma because the ependymal gap junctions permit unrestricted diffusion whereas the tight junctions in CP do not.

Consistent with high-level transport, the typical choroid cell has numerous mitochondria, a rich Golgi apparatus, and extensive microvilli at the CSF face. Basally located are elaborate infoldings and interdigitations as in the proximal tubule. Choroid cell organelle ultrastructure reflects brisk metabolic and transport activity linked with CSF secretion.

5.2. Ependymal Cell Lining: A Specialized Monolayer

Embryologically, the ependyma begins as a layer of spongioblasts lining the neural tube. In late fetal stages, the ependyma becomes multilayered, attaining a thickness of six or seven layers. In neonates, the lining

attenuates to two or three layers. In early postnatal life, ependymal-like tanycytes send long processes from their bases into the neuropil. By adulthood, the tanycytes largely disappear and the ependyma becomes a single layer of cuboidal or columnar cells.

Even within species, the adult ependymal lining is not structurally uniform. Great variations in morphology occur, especially in the third ventricle where ependyma intimately associate with the hypothalamus and subcommissural organ. Emanation of cilia from the apical surface is common. Specialized regions of the ependymal wall contain *tight* junctions between cells. Most ependymal cells, however, have *gap* junction structures intercellularly. Gap junctions do not completely envelop cells. Accordingly, intercellular clefts are permeable to macromolecules. Therefore, the functional hallmark of ependyma is permeability to most ions and molecules. A drug or endogenous substrate in CSF (6) can easily penetrate the ependyma to reach neurons and glia.

5.3. The Pia-Glia Membrane and Other Meningeal Tissue

The thin delicate pial membrane resembles ependyma more than CP. Discontinuous gap junctions between pial cells allow bidirectional exchange of solutes between subarachnoid CSF and subpial space. Large protein markers such as ferritin and horseradish peroxidase move readily across the pia to penetrate subpial tissue. The underlying *glia limitans* however restricts diffusion. On the other hand, Virchow-Robin spaces (perivascular cuffs of pia-glia and arachnoid that envelop major vessels penetrating the brain) promote uptake of materials via bulk flow from SAS to deep brain.

Pia mater along with the arachnoid is designated leptomeningeal tissue. A *thin* connective tissue membrane, the pia hugs the contours of brain and carries blood vessels. Arachnoid, on the other hand, is a *multilayered* avascular membrane between pia and dura maters. Arachnoid is separated from overlying dura by the subdural space and from underlying pia mater by SAS. Dura mater has venous sinuses into which CSF is cleared.

6. CIRCUMVENTRICULAR ORGANS OUTSIDE BLOOD-BRAIN BARRIER

The ventricular wall “houses” several organs with similar structure but distinct interrelated functions (25). These small organs are circumventricular (i.e.,

surrounding the ventricles). They include area postrema (AP), subfornical organ (SFO), pineal gland (PI), median eminence (ME), organum vasculosum of the lamina terminalis (OVLT), subcommissural organ (SCO), and the neural lobe of pituitary (NLP) (Fig. 6). Neurohypophysis (NH) refers to both ME and NLP. Unlike most CNS regions, circumventricular organs (CVOs) have highly permeable capillaries permitting diffusion of polypeptides into circumscribed, highly specialized regions of brain. Most CVOs readily receive macromolecular chemical “signals” from blood. Such humoral signals integrate neuronal pathways that mediate fluid/electrolyte homeostasis.

Several CVOs bear intimately to the diencephalon. A typical CVO has an ependymal interface and a highly permeable capillary interface. Collectively, the ependymal and capillary surface areas of CVOs are small (i.e., about 1% of the ventricles and brain capillary bed, respectively). Even with diminutive

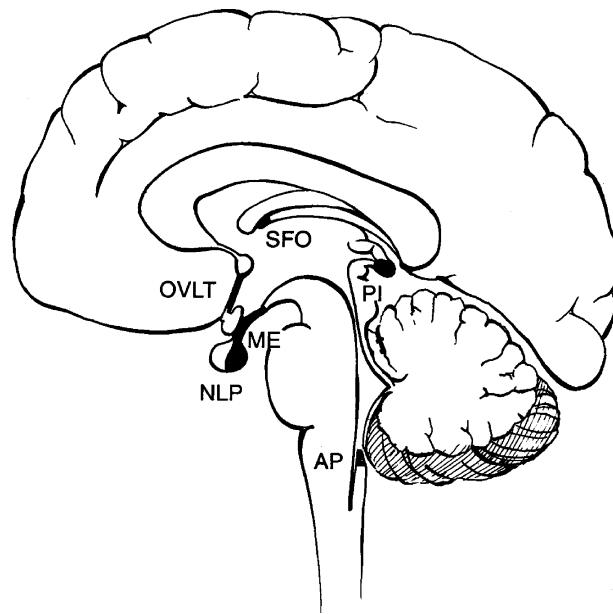


Fig. 6. Sagittal view of anatomic relationships among circumventricular organs (CVOs) located on the brain midline. CVOs are situated at strategic positions on the surface of the cerebroventricular system to perform neuroendocrine functions. The diminutive CVOs are highly vascular and have a variable number of neurons. Generally not protected by a BBB, the CVOs therefore contain central receptors for peripherally circulating factors (e.g., peptides). Neuronal processes extend into large perivascular spaces of CVOs. Each CVO is encompassed by a ring of glia (tanyctyes) with tight junctions that isolate the CVO from surrounding brain. Area postrema (AP) and subfornical organ (SFO) attach to CPs, with which they shunt blood. ME, median eminence; PI, pineal gland; OVLT, organum vasculosum of lamina terminalis; NLP, neural lobe of pituitary.

transport interfaces, the CVOs coupled with neuropeptide systems (e.g., angiotensin and AVP) importantly maintain fluid balance in the brain and whole organism (Table 4).

CVOs are categorized three ways. First, the “parenchymal” CVOs (e.g., SFO and AP) have dominant vascular inputs and neuronal outputs (Fig. 7A). Second, the “neurohumoral” CVOs or “gates” (e.g., the ME and OVLT) have substantial neuronal inputs and vascular outputs (Fig. 7B). Third, the “ependymorgans,” exemplified by SCO, have an intact BBB

but extensive communication with CSF. The major communication of “ependymorgans” seems to be active apical release of solutes into the ventricles and possible absorption of peptides from CSF.

CVOs have structural and functional connectivity. The SFO integrates water balance via angiotensin signaling. SFO communicates with other CVOs. AP is physically contiguous with fourth ventricle CP, the latter resembling a CVO. Lateral ventricle CP blood flow is markedly altered when AP is stimulated (26), suggesting CVO modulation of CSF formation.

Table 4
Functions and Anatomic Associations of Some CVOs

Organ	Location	Projections	Functions
Subfornical organ (SFO)	Attached to anterior dorsal wall of third ventricle, between the interventricular foramina of lateral ventricles.	<i>Afferent:</i> Central input is poorly characterized but is probably significant. <i>Efferent:</i> Projects into the preoptic area and hypothalamus (e.g., paraventricular and supraoptic nuclei).	Induction of drinking behavior, mediated by angiotensin signals; SFO can modulate fluid homeostasis by many mechanisms through multiple projections to endocrine, autonomic, and behavioral areas of the CNS.
Area postrema (AP)	Lies at caudal extent of fourth ventricle on the dorsal medulla in contact with the nucleus of the solitary tract.	<i>Afferent:</i> Input from underlying nucleus of the solitary tract and the dorsal motor nuclei of vagus; hypothalamus also innervates the AP. <i>Efferent:</i> Projections to major relay nuclei for ascending visceral sensory information; major projection to the parabrachial nucleus of the pons.	Modulates interoceptive information that reaches it through visceral sensory neurons or humorally by way of its permeable capillaries; directly affects motor outflow of the dorsal motor nucleus; stimulation of a chemotaxic center causes vomiting.
Organum vasculosum of the lamina terminalis (OVLT)	Lies in the anterior ventral extent of the third ventricle along the lamina terminalis.	Connectivity of the OVLT is poorly understood but seems to have a greater afferent input than efferent outflow.	Implicated in water balance because damage to it and surrounding structures affects drinking behavior and vasopressin release.
Median eminence (ME)	Forms the ependymal floor of the third ventricle in the central portion of the tuber cinereum in the hypothalamus.	<i>Afferent:</i> ME receives neuronal input from the arcuate nucleus and medial areas of preoptic hypothalamus. <i>Efferent:</i> No efferent projections to the brain; portal circulation carries hormones to the anterior pituitary.	Represents the final common pathway for the neural control of hormone production in and secretion from cells of the adenohypophysis (e.g., anterior pituitary).

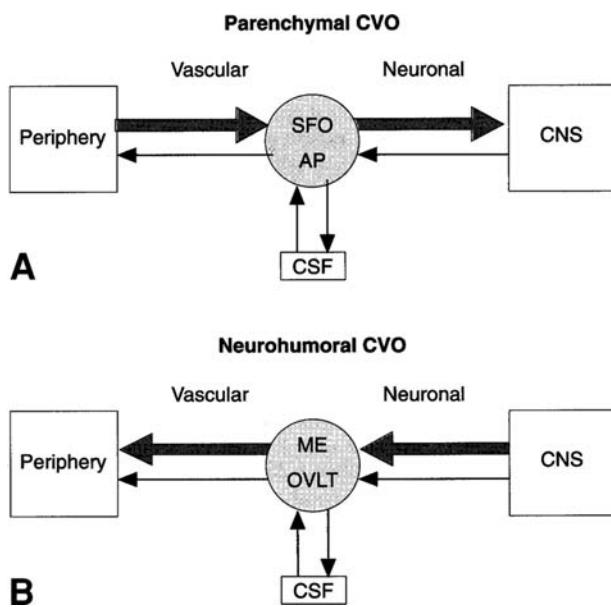


Fig. 7. Schema of the vascular, neuronal, and ependymal components of circumventricular organs (CVOs). (A) Subfornical organ (SFO) and area postrema (AP) receive a prominent vascular *input*, whereas (B) median eminence (ME) and organum vasculosum of lamina terminalis (OVLT) have a substantial vascular *output*. Neuronal *output* is strongest in SFO and AP, whereas neuronal *input* is substantial in ME and OVLT. On the other hand, SCO has relatively weak vascular and neuronal connectivities but a significant secretory and reabsorptive communication with CSF.

Complex anatomic connections of the CVOs with each other, and with the pituitary and autonomic nervous system, enable these CSF-adjacent organs to modulate neuroendocrine processes that stabilize the *internal milieu*.

7. ELABORATION OF CSF

Fluids are generated at multiple sites in adult CNS. The main source is CP. Extrachoroidal sites of production include a CSF-like secretion by the cerebral capillary wall and the metabolic generation of water by brain glucose oxidation. Because CP is the preponderant production site, representing 75% or greater of the total fluid formed, it is customary to regard true CSF as choroidal in origin.

CSF is not a passive filtration of fluid across membranes at the blood-CSF barrier (BCSFB). It is an *active* secretion by CP epithelium. The high rate of secretion of CSF (i.e., about $0.5 \text{ mL min}^{-1} \text{ g}^{-1}$ CP) depends upon a brisk vascular perfusion of the plexus. Blood flow to CP is about $5 \text{ mL min}^{-1} \text{ g}^{-1}$ (i.e., 10-fold faster than mean cerebral blood flow (CBF)). A ruddy color is imparted to CP by a large content of blood.

CSF is continually replenished. At least thrice daily, the normal volume of 140 mL turns over. NMR studies of humans reveal that CSF production increases at night. The net production of approximately 0.35 mL/min in man results in a 24-hour formation of about 500 mL in adults.

The initial step in CSF secretion is plasma filtration across CP capillaries. Fenestrated endothelium does not impede macromolecule movement across the capillary wall into CP interstitium. Furthermore, the choroidal interstitial space offers minimal restriction to diffusion of ions and substrates for transporters at the epithelial base. Several ion transporters have been identified. Their vectorial properties are schematized in Fig. 8.

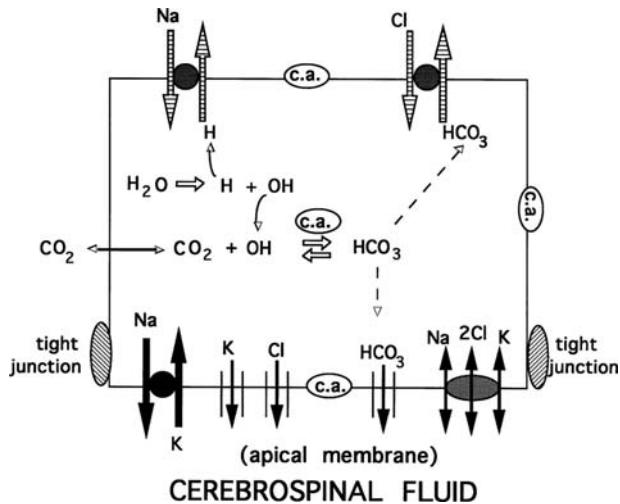


Fig. 8. Schema for ion transport processes in CP that underlie CSF secretion: There is coordinated activity of ion transporters and channels in the apical and basolateral membranes of CP that allow vectorial transport of Na⁺, K⁺, Cl⁻, HCO₃⁻, and water from epithelium to CSF. Membrane active transporters are depicted as *circles* or *oval*. Arrows indicate direction of transported ions. Antiporters (exchangers) are *arrows with horizontal lines*. The primary driving force for CSF secretion is Na-K pumping in the apical membrane, which keeps the choroid cell [Na] much lower than extracellular fluid [Na]. As a result, there is an inwardly directed Na gradient that promotes secondary active transport in the basolateral membrane. Na is taken up by epithelium in exchange for cellular H ion, and Cl for HCO₃. K, Cl, and HCO₃ (generated from carbonic anhydrase (c.a.) catalyzed hydration of CO₂) exit the cell via apical channels. Water movement across apical membrane aquaporin 1 channels is intimately associated with active ion transport. Na, K, and Cl are also extruded by a co-transporter (symporter). This unified model is for transport data from amphibians (Zeuthen T, *J. Physiol.* 1991; 444: 168) and mammals (Johanson C and Murphy V, *Am. J. Physiol.* 1990; 258: F1544).

Mammalian CSF is distinctive for a relatively low concentration of protein and certain organic substrates. Total protein concentration in adult CSF is 2 to 3 orders of magnitude lower than that in plasma. Glucose and urea concentrations are held at concentrations 60% to 70% of plasma. Many amino acids in CSF are at 10% to 20% of concentrations in plasma. This is due to transporters that actively remove amino acids from CSF.

The pH of CSF (7.35) is slightly more acidic than that of arterial blood (7.40) because CSF PCO₂ is higher than arterial PCO₂. Osmolality is greater than that of plasma by a few mOsm/L, due to the relatively high concentrations of Na and Cl in CSF. Normally CSF is a crystal-clear, colorless fluid. Colored is pathologic. Composition of human CSF is remarkably similar to that of other mammals.

7.1. Formation of Cerebrospinal Fluid

The main constituents of CSF are Na, Cl, and HCO₃. Knowledge of ion transport across CP enlightens understanding of CSF production. The apically-located Na-K pump (ATPase) has a pivotal role (Fig. 8). Primary active pumping of Na from choroid cells to CSF keeps intracellular [Na] low (20 to 30 mM) compared with the interstitial [Na] of 140 mM. A substantial inwardly directed transmembrane gradient for Na is the driving force for secondary active transport of Na into the cell by basolateral Na-H exchange. Coordinated activity of basolateral Na uptake and apical Na extrusion (Na pump and NaK2Cl co-transport) ensures continual net flux of Na across CP to CSF. K moves down its electrochemical gradient, from choroid cell to CSF. By way of aquaporin 1 channels, water accompanies the outward flow of CP ions into ventricles.

With respect to anion movement, Cl uptake by CP occurs by secondary active transport. Inward transport of Cl across the blood-facing membrane is driven by exchange with intracellular HCO₃. HCO₃ is amply generated from CO₂ hydration catalyzed by carbonic anhydrase. Cl and HCO₃ move into ventricular CSF via channels and transporters such as NaK2Cl symport (Fig. 8). Net transcellular movement of ions is fueled by ATP (hydrolyzed by ATPase), providing energy to create the Na gradient that drives transporters. Figure 8 recapitulates transporters that form the CSF.

7.2. Composition and Homeostasis of CSF

7.2.1. NASCENT FLUID

Newly formed fluid from the apical surface of CP epithelium has a high Na (158 mEq/kg H₂O), Cl (138), and HCO₃ (25) and lower concentrations of K (3.3), Ca (1.7), and Mg (1.5). Because of active transport, CSF Cl concentration is greater than that of plasma. CSF [K] is 1 to 1.5 mEq/kg H₂O less than in plasma. Nascent CSF levels of Ca and Mg, respectively, are held slightly lower and higher than corresponding normal concentrations in plasma. Stability of CSF [K] and [Ca] is essential because small deviations alter CNS excitability. Table 5 relates ionic concentrations in various fluids.

7.2.2. MIXING OF CHOROIDAL SECRETION WITH BRAIN INTERSTITIAL FLUID

CSF is modified as it flows from its origin in the CNS interior to distal SAS on the exterior of brain and cord. Nascent and cisternal CSF are compared in Table 5. Small concentration differences occur because the relatively permeable ependymal and pial linings permit exchange of CSF with brain ISF. The source of ISF is a slow, steady secretion by the cerebral capillary wall (i.e., astrocyte-endothelial

Table 5
Concentrations (mEq/kg H₂O) of Ions in Fluids Derived from Plasma

Fluid*	Cl ⁻	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺
Plasma	132	163	4.4	2.62	1.35
Plasma ultrafiltrate [†]	136	151	3.3	1.83	0.95
Choroid plexus fluid (nascent CSF)	138	158	3.28	1.67	1.47
Cisterna magna fluid	144	158	2.69	1.50	1.33

*Fluids were collected from cats (Ames A, Sakanoue M, Endo S. *J. Neurophysiol.* 1964; 27: 674).

[†]Plasma ultrafiltrate data, obtained from dialysis experiments, are values expected if the CSF were formed by passive distribution phenomena rather than by an active secretory process in the choroid plexus.

Table 6
Regional Differences in the Concentrations of Proteins at Various CSF Sampling Sites

Protein	Ventricular (n = 27)	Cisternal (n = 33)	Lumbar (n = 127)
Total protein	25.6 ± 1.1	31.6 ± 1.0	42.0 ± 0.5
Albumin	8.3 ± 0.5	12.7 ± 0.7	18.6 ± 0.6
IgG	0.9 ± 0.1	1.4 ± 0.1	2.3 ± 0.1

Values are means ± standard errors, given in units of mg/dL. The data demonstrate a gradient of protein concentration from ventricular to spinal fluid. Protein concentrations in CSF are 2 to 3 orders of magnitude less than that in plasma. Newly secreted CSF has a protein concentration of about 10 mg/dL. A CSF protein content of >500 mg/dL can indicate a lesion that is blocking the SAS.

complex) of a fluid similar to CSF. In addition, the exchange of ions and nonelectrolytes across the external limiting membranes of neurons and glia contributes to ISF composition.

Protein concentrations vary within CSF. There is an approximate twofold difference among regions. Lumbar CSF has about twice the IgG and albumin as ventricular fluid (Table 6). Such differences reflect regional variations in secretory and reabsorptive phenomena at the BCSFB. Still, the comparable compositions of CSF and ISF ensure that, even after mixing, CNS extracellular fluid has a characteristic if not uniform composition. In other words, as CSF flows down the neuraxis and exchanges with brain, the content of protein and ions in cisternal CSF, spinal fluid, and ISF, although altered, still closely resembles the nascent ventricular CSF rather than plasma.

7.2.3. REGIONAL SAMPLING OF CSF AND ISF

Sampling CSF or brain ISF sheds light on the neuronal extracellular environment. Experimental and clinical sampling sites include nascent CSF, large-cavity ventricular CSF, cisternal and lumbar fluids, and brain ISF. Nascent CSF exuding from CP is collectable by pipette, but complex surgery is necessary to isolate the plexus. CSF sampled from lateral, third, and fourth ventricles is a mix of nascent CSF and ISF percolating across the ependyma. It can be procured by invasive but limiting stereotactic procedures. Common sampling procedures include removal of subarachnoid CSF from cisterna magna (i.e., animal models) and the lumbar region (i.e., human spinal taps).

Experimental neuroscience has benefited from recent technical advances in microprobe dialysis. A tiny probe is inserted into a discrete region for continuous collection of brain ISF as a dialysate. Microdialysis is fruitful for assessing microregional differences

in neurotransmitter concentrations. Microprobes placed in cisterna magna to analyze CSF do not disturb brain or significantly alter CSF volume (27).

Whereas relatively small regional differences in CSF or ISF concentrations exist for inorganic ions, urea, and glucose, fairly large differences in regional concentrations occur for neuropeptides secreted by specific cell groups. A relatively high titer of angiotensin is found in hypothalamic ISF and in nearby third ventricle CSF; the elevated concentration of secreted peptide dissipates with increasing distance from hypothalamus.

7.2.4. HOMEOSTASIS OF CSF COMPOSITION

The hallmark of CSF is stability of solute composition in the face of excesses or deficiencies in plasma. CSF ion homeostasis is critical because small alterations in CSF [K], [H], [Mg], and [Ca] affect respiration, blood pressure, heart rate, muscle tone, and emotional state. CSF composition has been extensively analyzed after acute and chronic perturbations in systemic acid-base parameters and ion concentrations. Analyses of nascent and cisternal CSF samples reveal an impressive ability of CSF to maintain levels of K, Mg, Ca, and HCO₃ ions when challenged with plasma fluctuations. CP plays a major role in ensuring these *minor* changes in CSF ion concentrations. Water-soluble vitamins B and C are maintained during vitamin deficiency (4, 9). Even lipid-soluble vitamin E concentration is regulated in CSF (9).

Two factors undergird the ability of the blood-CSF interface to stabilize ventricular fluid in the face of vascular biochemical oscillations. First, the CP tight junctions act as a structural barrier that thwarts bidirectional diffusion of substances between blood and CSF. Second, many ionic and molecular transporters enable the plexus to regulate solute passage. CSF homeostasis thus involves finely controlled movement of solutes by active transport or facilitated diffusion not directly requiring energy.

In hypovitaminosis C, low levels of ascorbate in plasma are scavenged by an active transporter in the blood-facing membrane of CP (4, 24). Vitamin C concentrated in the cytoplasm moves out of the cell by facilitated diffusion across the CSF-facing membrane. Coordinated transport by these mechanisms at opposite poles of the cell, working in series, enables the CSF to concentrate vitamin C fourfold above that of plasma. Moreover, the basolateral active transporter for ascorbate is one-way into the cell. Therefore, vitamin C is not leached from CSF when the plasma level is severely reduced. Comparable mechanisms for other micronutrients (4, 9), also dependent on plasma substrate concentration and CP carrier affinity, ensure stable CSF concentrations.

7.2.5. NEUROHUMORAL REGULATION OF CSF SECRETION

Neurotransmitters and neuropeptides modulate choroidal secretion of ions, water, and proteins. In CP there is a great density of receptors for norepinephrine (α and β), serotonin (5-HT_{1c}), angiotensin II (AT₁), and vasopressin (V_{1a}). These receptors localize to the vasculature and choroidal epithelium. In cultured CP cells, serotonin stimulates secretion of transthyretin, a quantitatively important protein that transports thyroid T₄ across the blood-CSF interface. There is no evidence, though, for neurohumoral modulation of CP secretion of proteins to adjust CSF viscosity.

In the *in vitro* CP with no blood flow, exogenously applied serotonin, vasopressin, and angiotensin inhibit Cl⁻ release from epithelial cells into artificial CSF bath. Because Cl⁻ transport from the *in situ* plexus to ventricles is integral to CSF formation, the *in vitro* findings agree with known effects of neurohumoral agents to reduce CSF formation rate. Neuropeptides administered *in vivo* also curtail CSF formation, in part by reducing blood flow to CP (28). Markedly reduced perfusion of CP limits delivery of water and ions to the secreting epithelium.

Sympathetic nervous activity also “tones” the CSF secretion (Fig. 9). The superior cervical ganglia send adrenergic fibers to CP. Upon resecting the sympathetic fibers, there is increased CSF formation. This indicates a baseline inhibitory sympathetic tone. When such “braking action” is released by blocking sympathetic signals, fluid output is enhanced. Denervation findings have been bolstered by pharmacologic analyses establishing that α and β adrenergic agonists inhibit CSF production.

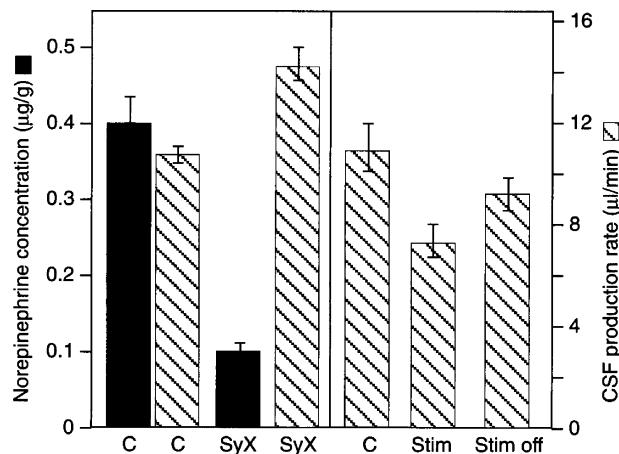


Fig. 9. Regulation of CSF formation by the sympathetic system: The involvement of sympathetic nerves in rabbit CP norepinephrine concentration (bars with solid black fill) and CSF production (bars with diagonal lines) was demonstrated from denervation (left) and electrical stimulation of nerves (right). One week after sympathetic denervation (SyX) of CP, there was a substantial decrease in norepinephrine concentration concomitant with significantly increased CSF formation compared with nondenervated controls (C). Electrical stimulation (Stim) of both superior cervical ganglia (which sympathetically innervate CP) significantly reduced CSF production rate. After stopping stimulation (Stim off), there was normalization of CSF formation. Bars are means \pm SE. (From Nilsson C et al., Brain Res Rev 1992; 17: 109–138.)

7.2.6. PHARMACOLOGIC MANIPULATION OF CSF FORMATION RATE

The clinical need for selective agents to lower ICP spurs research to find drugs to curtail CSF production. Agents from several classes have been used to assess dose-response phenomena in the CP-CSF. Acetazolamide, an inhibitor of carbonic anhydrase abundantly present in CP, consistently reduces CSF formation rate by 50% to 60%. Acute usage of acetazolamide is therapeutically effective in unloading augmented CSF pressure, but chronic use causes undesirable systemic acidosis. Cardiac glycosides such as ouabain markedly reduce CSF production by inhibiting Na-K-ATPase but have limited therapeutic value because of poor access to CSF and their ability to raise CSF [K]. Amiloride, by inhibiting Na-H exchange in CP decreases CSF formation appreciably if hefty doses (75 to 100 mg/kg) are employed. Other diuretic agents slow down CSF formation but introduce the expected complications consequent to urinary loss of water and electrolytes. More efficacious agents are needed to complement the moderately-effective acetazolamide.

8. INTERACTIVE BLOOD-CSF AND BLOOD-BRAIN INTERFACES IN MILIEU STABILIZATION

The epithelial BCSFB and endothelial BBB concertedly stabilize the fluid composition and volume (Fig. 10). Therefore the neuronal microenvironment depends upon material transfer at the two interfaces. Each barrier has distinctive transport and permeability features. The integrity of *both* barriers is essential for CNS extracellular homeostasis. Disrupted barrier function, individually or in tandem, harms brain parenchyma through altered fluid composition or pressure. Coordinated flow of solutes and water across the blood-CNS interfaces thereby optimizes the neuronal environment.

8.1. Biochemical Composition of Brain ISF

CSF formed by CP and ISF manufactured by capillary walls are not simply plasma ultrafiltrates. Rather, the finely controlled fluids generated across these barriers are active secretions. CSF and endothelial-derived fluid mix (Fig. 11). Bulk flow and diffusion promote mixing, depending upon the direction and magnitude of hydrostatic pressure or solute concentration gradients. Fluid mixing is substantial at ependymal and pial membranes. Solute and water movements between CSF and brain are bidirectional.

With ventricular fluid as the reference, CSF is either a “source” or “sink” for brain (Fig. 12). Brisk

secretion by CP furnishes generous amounts of Na, Cl, Ca, vitamins B, C, and E (and other micronutrients), transthyretin, IGF-II, prolactin, leptin, BDNF, and other neurotrophins to ventricular fluid. Collectively, this is a CSF supply “source” for brain. On the other hand, nascent CSF is normally low in protein and catabolites. Consequently, as ventricular fluid sweeps down the neuraxis, it acts as a drain or “sink” to remove harmful proteins and catabolites at higher concentration in ISF (due to metabolism and BBB leaks) than in CSF. Overall, ISF composition is influenced by transependymal fluxes of solutes, the gradients for which are impacted by transport activity at CP and BBB.

8.2. Stability of Brain Volume

Regulation of brain water content, hence volume, is critical for maintaining ICP within tolerable limits. Brain volume is affected by many parameters. Influx of water across the BBB and BCSFB interfaces importantly determines water balance among CNS compartments. Brain volume is stabilized at two levels: (1) interstitial (extracellular) fluid, and (2) neuronal and glial intracellular fluid (ICF).

8.2.1. INTERSTITIAL VOLUME

ISF volume is mainly the extracellular water content. It comprises 15% to 20% of brain weight. Water is transported across the relatively impermeable cerebral capillary wall slower than it is convected across

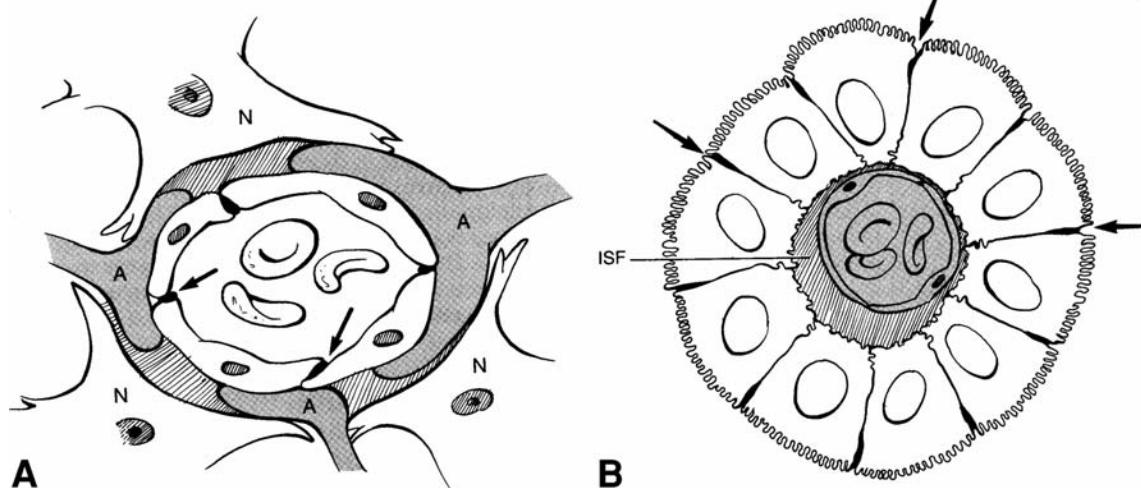


Fig. 10. Parenchymal cells of blood-brain and blood-CSF barriers **(A)** Highly idealized schema for components of BBB. Endothelium of cerebral capillaries lack fenestrations and are tightly joined by *zonulae occludentes* (arrows). Astrocyte foot processes (A) extensively abut the outside endothelial surface. Darkened area is the interstitial space surrounding the capillary wall. N, neuron. **(B)** Cross section of a choroidal villus. A ring of CP epithelial cells surround the ISF and adjacent vascular core. The basolateral surface has interdigitations, whereas the outer CSF-facing apical membrane presents an extensive microvilli system. Arrows point to tight junctions between cells at apical poles.

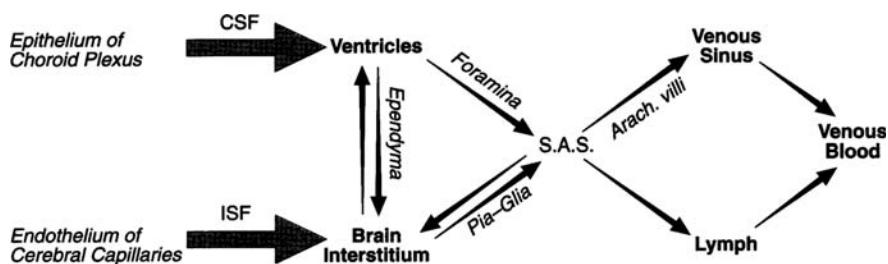


Fig. 11. Schema for fluid formation, exchange, and drainage routes in CNS: CSF is derived from constituents of plasma ultrafiltrate in the plexuses, by active secretion in the choroid epithelia. Plexus-generated fluid percolates down the ventricular system and then into the SAS of cisterna magna. From this great cistern, CSF continues to flow in the SAS overlying the hemispheres and cord. Finally, SAS fluid is reabsorbed into venous blood by a hydrostatic pressure-dependent mechanism in the arachnoid villi within dura mater, and into lymphatics via the cranial and spinal nerves. Simultaneous with CSF formation by CP is the slow production of cerebral ISF by brain endothelia. Once formed, ISF undergoes bulk flow exteriorly across the pia-glia membrane into SAS and interiorly into the ventricles, across the ependyma. Fluid flow is usually unidirectional through the ventricular foramina and arachnoid villi but is potentially bidirectional across the ependyma and pia-glia. For example, in hydrocephalus when CSF pressure is elevated, fluid moves from ventricles into brain tissue. The fluid in SAS is a mixture of CSF and ISF. Subarachnoid fluid drains into blood across arachnoid villi and via lymphatic tissue in the eyes and nose, which receive fluid draining along nerve roots. (Adapted from Audus KL, Raub TJ, eds. *Pharmaceutical Biotechnology*. New York: Plenum Publishing, 1993; 5: 467.)

permeable peripheral capillaries. Low hydraulic conductivity at the BBB is due to the high resistance at interendothelial tight junctions. It is debatable whether Starling's hypothesis, describing the role of

passive hydrostatic and osmotic pressure gradients for driving net filtration in many vascular beds, applies to fluid exchange between brain capillaries and surrounding ISF. Normally, the water gaining access to brain by slow permeation across capillaries eventually flows out via CSF. As a result, the pressure and volume of ISF are optimally maintained.

Water is also transported into CNS via CP with CSF formation. Most of the water generated in CNS originates from the four CPs. Once formed, CSF moves by bulk flow along pathways of least resistance (i.e., through the ventricles and SAS rather than through less compliant brain tissue). Consequently, the orderly flow of CSF and ISF along defined pathways keeps extracellular fluid volume and ICP stable.

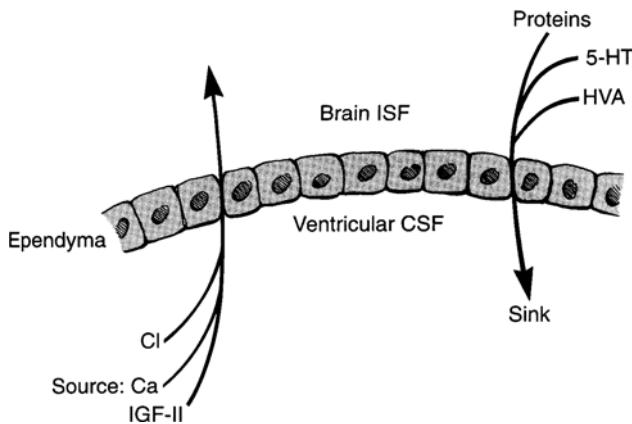


Fig. 12. CSF as a "source" or a "sink" for the brain. Depending upon the prevailing concentration gradient for diffusion across the ependyma, the CSF either supplies or removes solutes. CP secretes ions, proteins, and various micronutrients into ventricles. These transported solutes are derived either from plasma or the CP epithelium, which synthesizes certain proteins. Upon accessing the ventricles, the solutes are distributed by bulk flow of CSF acting as a supplier (or source) of materials for targeted cells in brain. Conversely, CSF acts as a drain or sink for solutes (metabolites of neurotransmitters, protein catabolites, iodide, etc.) that are break-down products of metabolism or leak across barriers from blood. Once in CSF, these potentially harmful materials are actively reabsorbed by CP or cleared from CNS by bulk flow drainage. IGF-II, insulin-like growth factor-II; 5-HT, 5-hydroxytryptamine; HVA, homovanillic acid.

8.2.2. INTRACELLULAR VOLUME

Brain volume intimately relates to water content. Cellular water content depends upon total osmotically active solutes. Neurons and glia continually exchange ions and organic molecules across external limiting membranes. Co-transporters move Na, K, Cl, inositol, and taurine into and out of cells, depending upon concentration gradients and transporter capacity. Consequently, water follows the net movement of transported solutes. In this manner, cell volume is stabilized even when extracellular tonicity is altered.

As brain tissue swells or shrinks in acute hyponatremia or hypernatremia, the activity of cellular co-transporters is upregulated or downregulated. Consequently, cell volume is rapidly reestablished.

Ischemic or pharmacologic disruption of cellular transporters swells the parenchyma, and thus the brain. Various states that alter the size of CNS intracellular and extracellular compartments are discussed below.

9. FLUID IMBALANCES: EFFECTS ON BRAIN AND CSF VOLUMES

Consideration of relative and absolute volumes of brain and CSF is essential to understand normal and deranged cerebral states. Water imbalance in brain affects the tortuosity (geometry of pathway winding) of extracellular channels. Such altered configuration of the interstitial space impacts excitability phenomena and signal transmission. Moreover, severely contracted ventricular volume (e.g., slit ventricle syndrome) may compromise the CSF sink and source functions. This is how brain and CSF volume changes affect neural activities.

A substantial increase in brain water content (i.e., edema) predisposes to herniation and intracranial hypertension. Edema may be generalized or localized around a tumor or infarct. In severe edema, the flow of nutrients to brain tissue and the orderly removal of unwanted catabolites are disrupted. In localized edema, tissue herniation can involve the cerebellar tonsils through the foramen magnum or the temporal lobe uncus across the tentorium. Edematous states are classified as affecting mainly the interstitium or cells.

9.1. Vasogenic Edema

The most prevalent brain edema, vasogenic, occurs in ischemia zones. Vasogenic edema is caused by increased permeability of the BBB, which allows plasma proteins and ions to leak across the endothelial wall. The resulting increase in ISF volume raises ICP, slows the EEG, and impairs consciousness. White matter is particularly affected. Vasogenic edema, frequent in head trauma and meningitis, is visualized by magnetic resonance imaging (MRI) or computed tomography (CT). Substantially increased brain volume from vasogenic edema occurs at the expense of the ventricles, which, because of CSF displacement, diminish to slits.

9.2. Interstitial Edema

Another distortion of the brain extracellular compartment is interstitial edema. Elevated ICP in obstructive hydrocephalus promotes spreading of ventricular water and Na into adjacent white matter.

Axial MRI reveals periventricular edema as a “white rim” around the frontal and occipital horns of the ventricles. Interstitial edema caused by chronic hydrocephalus is relieved by surgically shunting CSF to another body cavity. Hydrocephalus-induced interstitial edema is associated with enlarged ventricles.

9.3. Cytotoxic Edema

Cytotoxic edema is swelling of glia, endothelia, and neurons. As a result of expanded cell volume collectively, there is an attenuated interstitial space. Cell swelling results from drug poisoning, water intoxication, hypoxia from asphyxia, and acute hyponatremia. Under such conditions there is a net shift of water from extracellular space into brain cells. Cytotoxic edema can coexist with other forms of edema in encephalitis and meningitis. Brain swelling in severe cytotoxic edema diminishes the size of the ventricles and basal cisterns. Distorted ventriculo-subarachnoid spaces disrupt the CSF circulation. This alters homeostatic molecular exchanges mediated by CP-CSF.

10. CIRCULATION OF CSF

CSF is referred to as the “third circulation.” CSF derives from the anterior and posterior choroidal arteries. Choroidal venous drainage occurs largely by the vein of Galen. There are no lymphatic capillaries in CP or brain. However, the continuous flow of CSF through large spaces acts as a quasi-lymphatic system. Also, ISF percolates along low-resistance circumscribed pathways (e.g., around myelinated fibers) without lymphatic capillaries.

More than being clearance conduits, the CSF circulatory pathways distribute trophins and micronutrients from CP to brain parenchyma. Heading for multiple venous drainage sites, the human CSF flows through ventricles and SAS at about 0.35 mL/min. CSF movement is hampered by “upstream” clogging of CP (by deposits of Ca, immune complexes, and amyloid) and by “downstream” obstruction in the arachnoid (by fibrosis and amyloid). CSF disruption in neurodegeneration such as Alzheimer’s disease interferes with the renal-like function of CP-CSF. This exacerbates the primary disease.

10.1. Pressure Gradients

Several driving forces propel CSF. Formation of CSF by CP provides a hydrostatic pressure head of

about 150 mm of water, thereby generating a force for forward movement of newly formed fluid. Pulsation of blood in choroidal vessels promotes CSF circulation. Moreover, cilia beating at the apex of CP epithelium and some ependymal cells imparts additional thrust on ventricular CSF. The higher pressure of CSF relative to that in dural venous sinuses creates a positive gradient of 70 to 80 mm water that clears CSF by bulk flow from SAS to blood.

10.2. Direction of Current Flow

Ventricular fluid moves from lateral ventricles through the foramina of Monro down to the front of the third ventricle. After leaving the posterior third ventricle, CSF flow continues through the sylvian aqueduct eventually to empty into the fourth ventricle. CSF in the fourth ventricle seeps into SAS by three different apertures. Two exits are the bilateral foramina of Luschka at the extreme lateral portions of the fourth ventricle. The other major exit is the foramen of Magendie in the fourth ventricle roof. Here CSF empties into the *cisterna magna* or the *cisterna cerebello-medullaris*.

CSF flows from the base of the brain up over the hemispheric convexities until reaching the arachnoid villi in the walls of the superior sagittal sinus (Fig. 13). Thus CSF flows from the *cisterna pontis* to other cisterns: the *interpeduncularis* and *chiasmatis*, from which it sweeps upward over the hemispheric surfaces. It then progresses anteriorly upward along the longitudinal fissure, over the corpus callosum, along the sylvian fissure, and over the temporal lobes. At the most distal end of the flow route, the cranial subarachnoid CSF finally encounters the arachnoid villi (Fig. 13).

CSF also moves down from the *cisterna magna* to the posterior or dorsal surface of the spinal cord. CSF fills a sleeve of SAS around the spinal cord and even extends below the end of the cord into the region of the second sacral vertebra. Although the spinal subarachnoid CSF is in effect an anatomic “blind pocket,” nevertheless there is a slow mixing of spinal and cranial CSF induced by changes in posture.

10.3. Volume Transmission

Volume transmission is simply bulk flow or convection of CSF. Although CSF sloshes “to and fro” (with the cardiac cycle) in transit down the neuraxis, the *net* movement of fluid to distal drainage sites occurs as the result of hydrostatic pressure gradients from CSF to venous blood. Bulk flow distributes

materials *much faster* than by diffusion (1). Accordingly, CSF volume transmission enables efficient distribution of many peptides and hormonal signals along the CP-CSF-brain nexus. Moreover, volume transmission allows CSF to be “turned over” relatively rapidly and thereby helps to purify the CNS interior.

Convection of drugs via CSF importantly affects elimination half-time. Thus, CSF-borne methotrexate, an antitumor agent, has an excretion half-time similar to the CSF renewal rate of about 6 hours. Substantially reduced CSF turnover (as in aging, chronic hydrocephalus, and Alzheimer’s disease) compromises volume transmission. Such stagnated CSF flow enhances drug residence time in CNS and leads to pharmacotoxicity.

11. DRAINAGE OF CSF

CSF drainage is of neurosurgical interest because ICP problems attend inadequate fluid clearance from CNS. Normally, CSF drainage keeps pace with formation, and so ICP is stabilized. There are two bulk-flow mechanisms or pathways for CSF leaving the CNS. The lesser is valve-like drainage directly into cranial sinus venous blood. The other *more extensive* pathway is convective clearance via the cranial and spinal nerves into prelymphatic tissue spaces and then lymphatic nodes.

11.1. Arachnoid Villi and Granulations

Concepts about CSF reabsorption are under revision. For many decades, the prevailing view was that the arachnoid villus plays a prominent role in CSF egress to venous blood. Arachnoid villi and the compositely larger granulations increase more or less with mammalian species size. Historically important research by Welch, Davson, Pollay, Gomez, and Butler extensively described the anatomy and *in vitro* function of arachnoid villi; see the overview by Rapoport (29). Recent topographic assessments of human arachnoid granulations on the cortical surface (30) set the stage for functional studies of these granulations in patients with impaired CSF reabsorption.

11.2. Intracranial Arachnoid Villi and Granulations

In the traditional model, CSF returns to blood (passively along hydrostatic pressure gradients) across arachnoid villi into the dural venous sinus. A hydrostatic pressure gradient of at least 30 mm H₂O from CSF to blood enables large proteins and

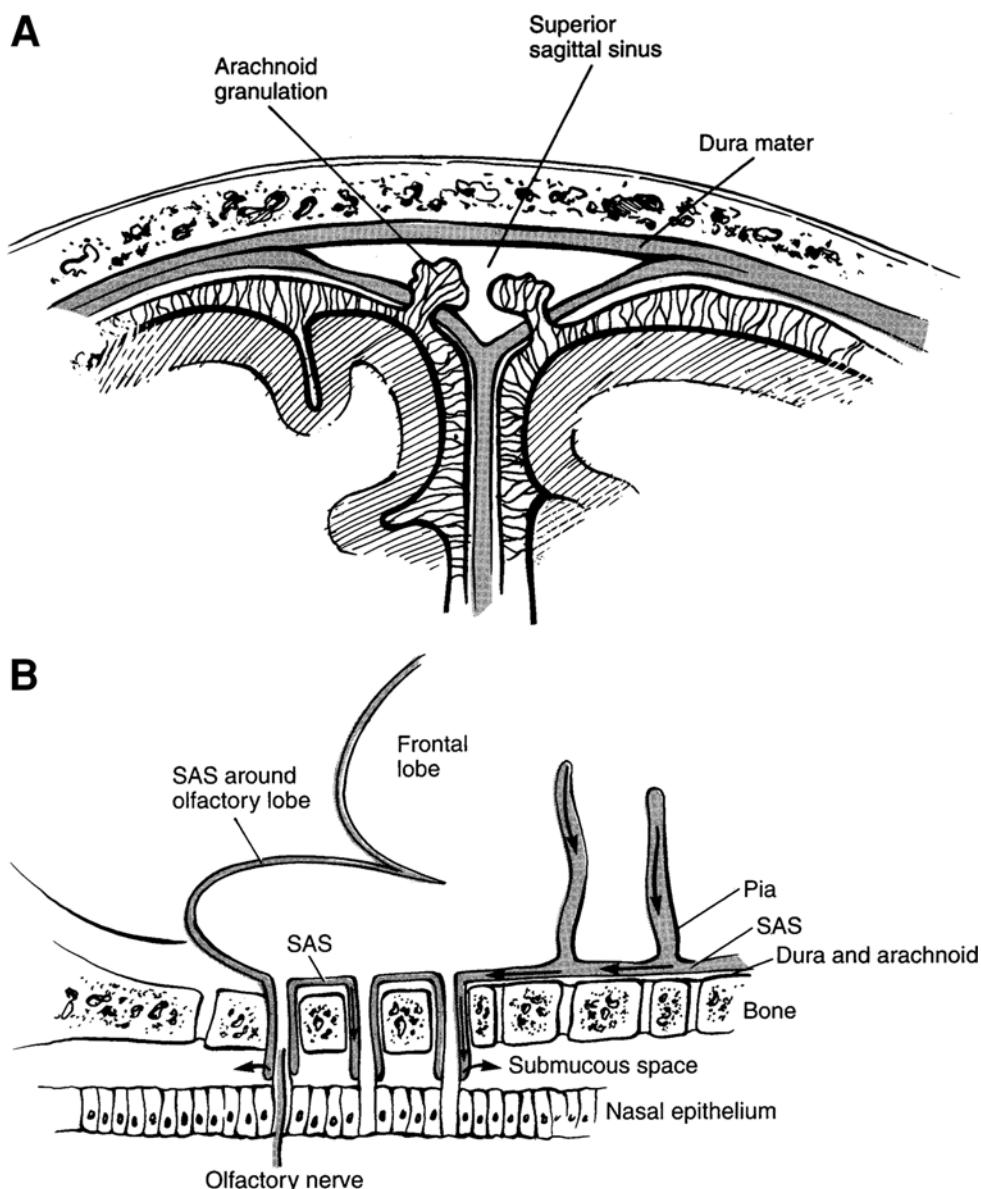


Fig. 13. CSF drainage by bulk flow across arachnoid villi in the dura mater and into lymphatic capillaries of eye and nose. (Top) A hydrostatic pressure gradient drives CSF in SAS into the venous sinus via valve-like structures in arachnoid villi. Valves are big enough to allow protein cells in CSF to pass, but normally only unidirectionally, from CSF to blood. Therefore once CSF-borne macromolecules have flowed into the venous sinus, they effectively have been cleared from CNS. Villi increase in size and number with advancing age. (Bottom) Another route for CSF drainage is along cranial nerves into submucosa of nose and eye. CSF flows via sleeve-like extensions of the SAS around the nerve, through the cribriform plate, finally to reach nasal submucosa. Lymphatic capillaries drain the submucous spaces and convey fluid to lymph nodes. (From Bradbury M et al., *Am. J. Physiol.* 1981; 240: F335.)

erythrocytes to penetrate the one-way valves that regulate outward CSF flow. At about 120 mm H₂O of CSF pressure, the rate of absorption equals CSF production. Conversely, when venous pressure exceeds ICP, the valves close and prevent blood reflux into SAS.

Vacuoles in the mesothelium of arachnoid villi suggest a dynamic pressure-sensitive vacuolation.

Vacuole formation in the valves likely constitute transendothelial channels for CSF flow. Such function may be particularly relevant when ICP is substantially elevated, as in congenital hydrocephalus. However, under normal ICP in most animals modeled, a relatively small proportion of CSF is convected across arachnoid villi.

11.3. Extracranial and Spinal Nerve Outflow Pathways

With respect to the preponderance of CSF drainage, the fluid must first pass through *lymphatic tissue* before reaching venous blood (Fig. 13). The systematic and elegant work of Johnston and colleagues over the past decade provides substantial evidence from several mammals that a large fraction of CSF outflow occurs across the cribriform plate and along the olfactory nerve into submucosal spaces (31). Sleeves of subarachnoid CSF surround the optic and olfactory nerves in particular as well as other cranial and spinal nerves (31–33). A positive-pressure gradient promotes CSF flow along the perineural space that extends into the submucosa of eye and nose. There the lymphatic capillaries reabsorb CSF and convey it to cervical lymph glands. CSF exiting by this route is thus first exposed to the immune system prior to reaching venous blood. Antigenic material (e.g., products of myelin breakdown) in outflowing CSF thereby induces antibody reactions that eventually affect the CNS via altered immune cell transport at the CP and BBB (34).

12. CSF PRESSURE-VOLUME RELATIONSHIPS

The adult skull is incompressible. Therefore ICP rises after a significant increase in any of the major constituents of the intracranial space: brain parenchyma, CSF, and vascular tissue. The CSF constituent is a potential liability as well as asset. A life-threatening increase in ICP results from CSF occlusion. On the other hand, shunting of CSF unloads ICP. CSF pressure is thus useful to evaluate or “titrate” therapeutic responses.

Markedly elevated ICP seriously injures the CNS. Normally, the CSF buffers impact forces and acute changes in vascular pressure. Tumors, infections, neurosurgery, trauma, and diseases, however, can elevate ICP. There are several sites for monitoring ICP and numerous treatment modalities to alleviate intracranial hypertension.

12.1. Normal Ranges of ICP

ICP is commonly assessed in the lumbar region. In patients with normal blood pressure and no pathologic lesions, the SAS pressure of reclining individuals is close to 100 mm H₂O. The typical range for lumbar CSF pressure is 50 to 150 mm H₂O or 4 to 11 mm Hg. CSF production is stable over the normal range of CSF pressure. Formation of CSF, however, may decrease when ICP is substantially elevated as in

severe hydrocephalus. Augmented CSF pressure reduces plasma filtration across choroidal capillaries (i.e., the initial step in fluid formation).

12.2. Measurement of ICP

Pressure on the intracranial contents can be probed at various depths under the skull. Probes are placed epidurally as well as in the SAS, brain parenchyma, or lateral ventricle. Figure 14 depicts monitoring sites. The paragon for measuring ICP is the intraventricular catheter connected to a manometer by a fluid-filled tube. To place the catheter, a burr hole is drilled over frontal cortex. Upon dural piercing, the catheter is directed into a lateral ventricle with the tip near the foramen of Monro. ICP assessments are made by ventricular probing with usually few complications (Table 7).

Relatively noninvasive pressure probes can be applied epidurally. Epidural pulsations, an indirect measure of ICP, are monitored by fiberoptic, strain gauge, or pneumatic systems. This approach lacks the accuracy to justify regular usage. Pressure recorded subarachnoidally over the convexities also estimates ICP. The subarachnoid screw or bolt is a hollow tube secured to the calvaria. The device is connected to a fluid-filled system with an external transducer. Intraparenchymal pressure recording guides ventricular cannula placement. Camino fiberoptics are used for intraparenchymal, epidural, and intraventricular monitoring. Infection rates for pressure-measuring systems are <1% if monitors are placed 4 days or less. Table 7 recapitulates various approaches.

12.3. CSF Pulsations and Pressure Waves

CSF pulsates due to oscillating arterial and venous pressures. Intracranial venous pressure decreases and increases, respectively, during respiratory inspiration and expiration. Altered venous pressure in the respiratory cycle transmits to ICP. Moreover, arterial systolic pulsations, particularly in CP, synchronously elevate the CSF pressure.

Sustained intracranial hypertension causes pathologic “plateau waves.” Unstable vasomotor control (e.g., loss of cerebrovascular autoregulation or reduced CBF) can trigger an onset of plateau waves. Clinically significant plateau waveforms may last 5 to 20 min and be associated with an ICP of >1000 mm H₂O. Elevated CSF pressure results from enhanced cerebral blood volume. Plateau waves occur in advanced stages of intracranial hypertension and often indicate CNS damage.

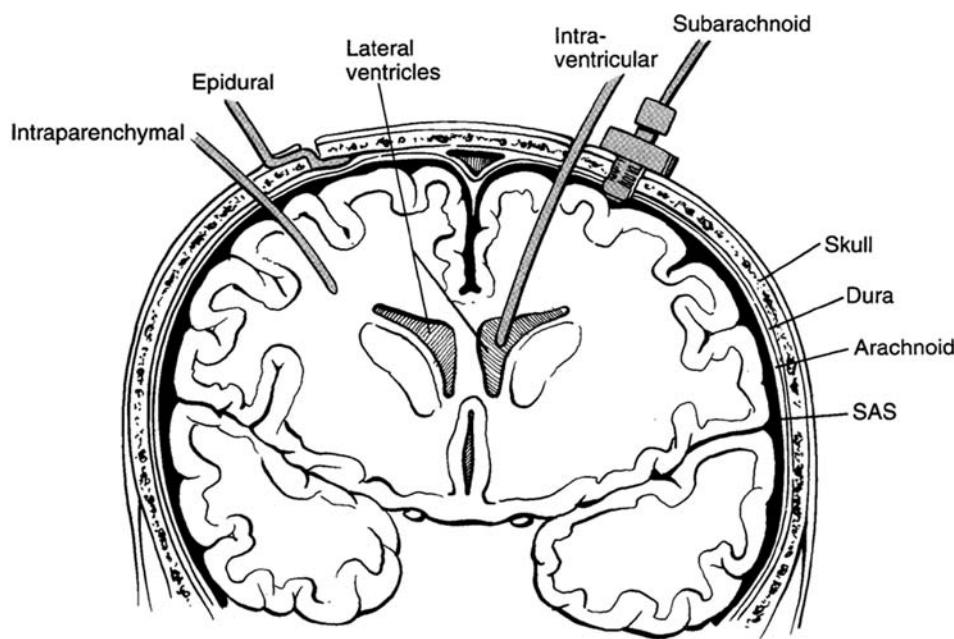


Fig. 14. Various sites for monitoring ICP. The intraventricular catheter is inserted through a burr hole in the frontal lobe, down into a lateral ventricle near the foramen of Monro. Placement of probe in the epidural space carries minimal risk for brain infection because the dura remains intact. The subarachnoid bolt is placed in SAS but often needs saline irrigation to remain patent. Intraparenchymal microtransducers are insertable for 2 to 3 cm into white matter. (From Lyons MK and Meyer FB, *Mayo Clin. Proc.* 1990; 65: 684.)

12.4. Relationship Between ICP and Cerebrovascular Parameters

Markedly elevated ICP thwarts arterial perfusion, causing irreversible damage. Cerebral perfusion pressure, the difference between mean systemic arterial blood pressure and ICP, is critical. When ICP rises to the systolic blood pressure, CBF ceases because the driving force for perfusion becomes negligible. If prolonged, brain death ensues.

Substantially elevated ICP compromises delivery of O₂ and nutrients to the brain. Because of skull

rigidity, the fixed volume of the intracranial space does not properly accommodate increased tissue mass (such as space-occupying tumors and blood clots) or edematous fluid secondary to trauma. In such cases, failure to adequately lower the ICP and maintain perfusion can be fatal.

12.5. Management of Elevated ICP

Trauma is a common cause of rising ICP. Cerebral edema or bleeding into CSF markedly elevates ICP. Surgical and nonsurgical means help to alleviate

Table 7
Devices and Locations for Monitoring ICP

Location	Advantages	Disadvantages
Intraventricular	Reliable measure of ICP; allows CSF drainage; good pressure waveform.	Invasive; risk of infection, need to enter ventricles; can obstruct.
Epidural	Less invasive, dura remains intact; less risk of infection.	No CSF drainage; poor measure of ICP; no waveform.
Subarachnoid	Brain theoretically not penetrated, lower risk of infection; ease of placement.	No CSF drainage; brain tissue easily obstructed; fluid-filled system but waveform usually poor.
Intraparenchymal	Non–fluid-filled system; fairly reliable measure of ICP.	Invasive; risk of infection; no CSF drainage.

CSF, cerebrospinal fluid; ICP, intracranial pressure. (Adapted from Lyons MK, Meyer FB. Cerebrospinal fluid physiology and the management of increased intracranial pressure. *Mayo Clin. Proc.* 1990; 65: 684; and from McComb JG [personal communication].)

intracranial hypertension. Surgical decompression is feasible in patients with hematomas located epidurally, subdurally, or parenchymally. Drainage via ventriculostomy is the prime method of lowering ICP elevated by obstructed CSF drainage.

When surgery is contraindicated, there are postural and pharmacologic strategies to unload ICP. Head elevation above the heart facilitates venous drainage. To minimize edema from injured cerebral vessels, fluid restriction is useful in the patient without *diabetes insipidus*. High levels of blood glucose should be avoided because the hyperglycemia of head injury is detrimental to cerebral function if ischemia is concurrent. Ventilatory support by hyperventilation rapidly lowers ICP in many patients. This benefit results from cerebrovascular constriction to lower blood volume.

Diuretic agents reduce CNS water content, thereby decreasing ICP. Furosemide and acetazolamide curtail the CP output of CSF into the ventricles and also act as renal diuretics to eliminate body fluid. Mannitol is used as an osmotic agent because it slowly permeates the blood-brain and blood-CSF barriers. Consequently, the osmotic gradient set up between blood and brain “pulls” water from nervous tissue. The dehydrating effect of mannitol may be prolonged by concurrently using loop diuretics. Because osmotic agent clearance from blood is faster than CNS, a “rebound” intracranial hypertension occurs if mannitol is not carefully administered.

Corticosteroids and barbiturates have limited use in controlling ICP. High doses of dexamethasone and methylprednisolone decrease ICP in some patients with large brain tumors by suppressing edema formation and stabilizing membranes near the tumor. Barbiturates such as pentobarbital are used when conventional modalities fail. Pentobarbital decreases CBF and cellular metabolism, thereby reducing ICP.

12.6. Compression of CSF and the Optic Nerve

Ventricular tumors directly occlude CSF drainage, with a resultant increase in ICP. Tumors distant from the ventricles may not significantly obstruct CSF flow until the mass becomes substantial. Tumor growths in the posterior fossa (e.g., in cerebellum) exert pressure on the fourth ventricle roof, thereby obstructing CSF flow into SAS. Brain tumors compressing the optic nerve cause papilledema by choking the optic disk. Sustained papilledema severely damages the optic nerve and can cause blindness. Papilledema also results from *pseudotumor cerebri* or benign intracranial hypertension. In this disorder, there is augmented CSF production and interstitial edema.

Benign intracranial hypertension elevates ICP in young obese women. It can be effectively treated over several weeks.

12.7. Hydrocephalus, Ventriculomegaly, and ICP

Hydrocephalus is enhanced CSF volume, with or without elevated ICP. *Compensatory hydrocephalus* occurs without augmented ICP. Here an expanded CSF volume compensates for cerebral atrophy in primary CNS disease. Another syndrome, *normal pressure hydrocephalus (NPH)*, results from chronically impaired absorption into blood. CSF composition and pressure are variably altered in NPH. Although ventricles enlarge, there may not be size changes in cerebral cortex or SAS. CSF shunting in selected adult NPH patients relieves the triad of unsteady gait, dementia, and urinary incontinence.

Hydrocephalus is either communicating or non-communicating (obstructive). In *communicating hydrocephalus*, there is an open system between ventricles and SAS. Hydrocephalus can be caused by altered CSF dynamics (production or absorption) or by obstructed flow of CSF through the SAS. In *obstructive hydrocephalus*, something impedes fluid percolation within the ventricles, aqueduct, or fourth ventricle outlets. With blocked CSF flow in developmental abnormalities, inflamed tissue, or tumors, the retained fluid in ventricles elicits a rise in ICP.

Continuously elevated ICP in hydrocephalic states with accumulating CSF causes ventriculomegaly. Enlarged ventricles compress underlying cells and their processes. During early hydrocephalus stages, there is damage to ependyma and periventricular white matter. Two to 3 weeks of severe hydrocephalus can compress the cortical mantle, sometimes to 25% of original thickness. Cytological and cytoarchitectural studies of brain cells in animal models reveal greatly shrunken somata and abundant vacuoles. In severe hypertensive hydrocephalus, there is a diminution of axons and blood vessels. Surgical shunting of CSF to the peritoneum reduces ventriculomegaly and decompresses cortex. With early shunting, much structural and functional damage is reversed. Shunting evidently facilitates removal of substances from ISF, the effect of which needs elucidation.

13. CELLULAR COMPOSITION OF CSF

A distinguishing feature of CSF, especially in relation to blood, is a paucity of cellular elements. CSF usually contains no more than four mononuclear cells

or lymphocytes per cubic millimeter. White cell counts of 5 to 10 per mm³ can signify pathology. Elevated cell counts in CSF occur after brain injury, central inflammatory processes, or tumor cell invasion. Cytologic examination of CSF is becoming delinquent with application of polyclonal and monoclonal antibodies to specify pathology.

13.1. Normal Conditions

The high water content of CSF (> 99%) reflects a low cell count. Normal CSF contains a few small B and T lymphocytes and monocytes. The relatively impermeable CNS barriers prevent significant penetration of blood cells into CSF of healthy humans. An elevated erythrocyte count in CSF can indicate blood contamination of the specimen. Sloughed choroid epithelial, ependymal, or arachnoidal cells in CSF samples are rare.

13.2. Infective States

CSF pleocytosis is common in acute infections of the CNS. In fungal infections, the predominant cell in CSF is the lymphocyte, whereas in bacterial infections it is the neutrophil. In acute bacterial meningitis, >90% of cells in CSF may be neutrophils. With severe infections such as a ruptured abscess, CSF cell counts exceed 20,000 per mm³.

The appearance of ependymal and choroidal cells in CSF, along with white blood cells, signifies neurologic diseases and infections. However, mumps virus uniquely causes ependymitis. Ependymal destruction leads to aqueductal narrowing and hydrocephalus.

Cellular composition of CSF in AIDS patients is highly variable. In one study of HIV-1, only a small percentage of patients developed lymphocytic pleocytosis in CSF even with fully acquired immunodeficiency. AIDS patients have many opportunistic infections. Therefore, CSF immune cell profiles will be similar to those caused by opportunistic invaders, although response magnitude may be blunted by immunodeficiency.

13.3. Neoplastic Diseases

CSF sampling is used to manage brain tumor patients. Primary neoplasms around the brain stem, cerebellum, and spinal cord can abut CSF pathways and shed tumor cells that appear as sediment in CSF samples. Medulloblastomas arising from the external germinal layer of cerebellum shed many tumor cells into CSF. In contrast, meningiomas shed few malignant cells. Meningiomas are firm arachnoidal

elements that do not readily exfoliate cells, thus the low frequency of CSF-positive specimens.

Metastatic neoplasms (carcinomatosis) have a greater propensity to exfoliate cells than do most primary tumors. Thus there is a high yield (20% to 50% positive) from cytologic examination of CSF malignant cells in cerebral metastases. Carcinomas of lung, stomach, and breast commonly metastasize to CSF. The less frequently occurring melanoma usually metastasizes rapidly to brain and CSF and is characterized by pigmentation. Occasionally, the CNS-CSF metastasis is present before the primary peripheral tumor is discovered. Inflammatory cells intersperse with tumor cells in CSF. When exfoliated tumor cells in CSF are sufficiently characteristic, the peripheral origin can sometimes be identified.

Complete treatment of leukemia depends on CSF cytologic analysis. Because chemotherapeutic agents do not readily penetrate the BCSFB and BBB, malignant cells inside CNS can remain after treatment. Because surviving tumor cells in the SAS are a reservoir contributing to systemic relapses, it is essential to pinpoint even a few leukemic cells in CSF. Flow cytometry combined with monoclonal antibody staining detects small numbers of specific malignant cells. Such CSF examination is important in leukemia management to decide upon radiation or intrathecal treatment.

14. CLINICAL USAGE OF CSF

Many neurologic disorders present with altered CSF chemistry. As brain metabolism is altered by disease or trauma, many cellular metabolites released into ISF gain access to CSF. Thus, CSF biochemical profiles often change during illness or injury. Such modified CSF composition often mirrors perturbed neurochemistry. Therefore, clinicians use CSF findings to guide diagnoses and management. Limitations to this approach need to be recognized. Nevertheless, if CSF samples are appropriately interpreted, the biochemical and cellular information is diagnostically valuable.

Lumbar SAS is a convenient region for sampling CSF, after the potential value and risks of tapping have been considered. Although CT obviates some punctures (e.g., suspected subarachnoid hemorrhage), there are many indications to procure CSF samples.

14.1. Diagnostic and Prognostic Benefits

Lumbar punctures are done mainly for diagnoses. CSF analyses can strengthen the diagnoses of

neurosyphilis, multiple sclerosis, and many inflammatory diseases involving meninges. Inexplicable seizures should also prompt the analysis of CSF biochemistry. In myelography, the CSF removed at the initial procedural stage should be characterized as a baseline for future comparisons. Access to CSF by way of a lumbar tap also permits measurement of pressure in clinical assessments of intracranial hypertension.

14.2. Intrathecal/Intraventricular Administration of Drugs

Drugs that cross the BBB and BCSFB too slowly for therapeutic usefulness can be administered intraventricularly (by lateral ventriculostomy) or intrathecally (by infusion into spinal SAS); see Fig. 15. Drug injection into human nervous tissue is usually not feasible. However, agents infused into CSF gain

access to the permeable ependymal and pial linings where diffusion into brain occurs.

CSF infusion circumvents blood-CNS barriers and exploits pharmacokinetic factors. Drug metabolism and protein binding are usually less problematic in CSF than in plasma. Central administration of drugs largely avoids renal/hepatic metabolism. Because CSF has a meager protein level, there is low probability that a drug effect will be diminished by extensive binding to a large central reservoir of extracellular albumin or other protein.

There is advanced technology of implantable pumps to deliver drugs via CSF. Some pumps hold 50 mL and deliver at 75 to 100 $\mu\text{L}/\text{min}$. Variation in delivery rate is $\pm 5\%$. Drug dose is regulated by adjusting reservoir concentration. Therapeutic agent stability is required for many weeks at 37°C in a CSF-like buffer. Vehicles are set to a certain pH and osmolality and cannot contain a solubilizer harmful to exquisitely sensitive brain.

A big plus for CSF drug delivery is localized pharmacologic effect. This is significant because many neurologic diseases are circumscribed. Central drug delivery is efficacious when there is optimal blending of pharmacodynamics and pharmacokinetics. Intrathecal morphine, used successfully in pain control, avoids systemic narcotic effects like anorexia and oversedation. Intraventricularly administered bethanechol (an acetylcholine agonist) has improved Alzheimer patients. Future investigations should reveal how higher brain center functions can be effectively modified with intraventricular infusions or CP-mediated delivery of therapeutic agents (6).

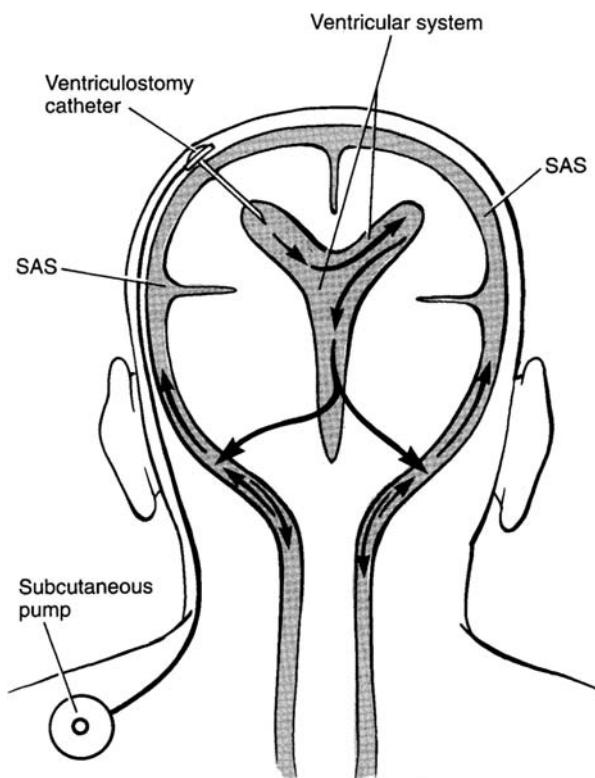


Fig. 15. CSF infusions of drugs through a totally implanted system for constant intraventricular drug infusion. Components of the system include a subcutaneously implanted Infusaid pump, Silastic catheter, and ventriculostomy reservoir and catheter. The amount of drug for refill is calculated as the product of dose rate times pump capacity, divided by flow rate of pump. SAS, subarachnoid space. (From Dakhil S, Ensminger W, Kindt G, et al. *Cancer Treatment Reports*, 1981; 65: 405.)

15. NEW OUTLOOKS FOR CSF TRANSLATIONAL RESEARCH AND THERAPY

Appreciation of the circulatory vitality of the CP-CSF-arachnoid nexus for brain metabolism is prompting novel investigations in basic and clinical neuroscience. In animal models, the burgeoning CSF dynamics in early development coincides with a brain growth spurt (3). Gene expression in the maturing CP is key to understanding the impact of CSF growth factors and neurotrophins on the SVZ. There, the birth and migration of neurons is pivotal for “architecturally” shaping the brain. Because of secretion of multiple growth factors at the BCSFB, the CP generates a “hotbed environment” for the brain interior (3, 5, 13). A disrupted flow of vitamins and modulating proteins in ventricular CSF substantially alters

fetal brain morphing (3, 35). Congenital hydrocephalus models demonstrate that disordered CSF dynamics has dire consequences on neuron generation in germinal matrix (35). To alleviate pediatric hydrocephalus, rational prophylactic measures (e.g., CSF growth factor manipulation) are on the horizon.

Experimentally, the BCSFB is gaining attention in regard to homeostatic and pharmacokinetic modeling. New insights strengthen the construct that CP makes major contributions to brain health (3, 16). Choroid epithelial cells not only *distinctively* transport plasma-borne micronutrients and particular hormones but also *uniquely* synthesize and secrete proteins such as transthyretin. Transport *specificity* at the BCSFB points to pharmacologic opportunities (6) for regulating the movement of neurotrophic and reparative agents to neuronal networks. Biomedical devices and strategies for stem cell augmentation (8), as well as CP transplants and transgene manipulation (6, 36), may also be feasible by intraventricular instillation (6). Boosting trophic factors, antagonists, and chelators in CSF will benefit neural repair in trauma (37), ischemia (38), hyperthermia (39), and infectious disease.

Another advance, with potential to expedite translational research, is appreciation that the BCSFB and BBB should *both* be incorporated in paradigms of metabolic and fluid balance (3). The multicompartmental nature of CNS dictates that experimentation should factor the CP functions along with the cerebral capillary transport phenomena (influx and efflux). Two examples are the translocation of water and amyloid peptides by aquaporins (3) and the LRP-1 transporter (3, 40), respectively. Conceptualizing *both* plasma and CSF as potential sinks (40, 44) on water and solutes will enhance pharmacotherapy to ameliorate hydrocephalus and Alzheimer's disease (3, 41).

Aging exerts a great toll on CP mechanisms that protect brain from systemic perturbations (42). In aging, free radicals and other oxidative products injure the choroidal epithelium, thus interfering with CSF production and solute homeostasis (16). The decline in CSF turnover rate with age places the brain at risk because the CNS does not receive adequate micronutrition (4) or properly remove catabolites (41, 43). Consequently, amyloid peptide fragments are retained in ISF and likely wreak havoc with cognitive neuronal networks and the stem cell environment. A worthy goal is to identify agents that stabilize the BCSFB and allow CP to maintain CSF formation in senescence. This would maintain CSF sink action (44) and the clearance of

harmful toxins (3, 41, 43). Otherwise, stagnated CSF dynamics in advanced aging predisposes to the dementia of chronic hydrocephalus (41). PET scans and refined MRI analysis (45) of CP-CSF status in the elderly will identify vital links among distressed CSF circulation, compromised cognitive abilities, and impaired conversion of stem cells to neurons.

Several salient questions await answers for the field of CSF dynamics. Can peptidergic agents (12, 46, 47) be used to therapeutic advantage in controlling CSF formation? To what extent does reduced CSF formation in aging (42) and neurodegeneration compromise ISF movement throughout brain and to drainage sites (48) and thus predispose to plaque formation (3, 41, 43)? In addition to bulk flow, do molecular transport and unconventional mechanisms facilitate CSF reabsorption by arachnoid (30, 49, 50)? Resolving these questions should enhance therapeutic regulation of the renal-like CP transporters (24) and arachnoid efflux systems, thereby sustaining CSF-brain throughout life.

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SELECTED READINGS

1. THE SPINAL CORD

Sensory information from the body is transmitted into the central nervous system (CNS) through the dorsal root projections into the spinal cord. Motor neurons in the spinal cord project their axons into the periphery to innervate muscles and autonomic ganglia. Somatic perceptions, coordinated movements, and autonomic functions depend on the integrity of the spinal cord and its projections. The neurons and fiber bundles within the spinal cord are organized in a

simpler and more uniform way than other parts of the CNS.

1.1. Segmental Organization of the Spinal Cord

During early embryonic life, the spinal cord extends almost the whole length of the vertebral canal. As development proceeds, the body and the vertebral column grow at a much greater rate than does the spinal cord. As a result, in newborns the spinal cord extends only as far caudally as the mid-lumbar vertebral levels and in adults (Fig. 1) only to the level of the first or second lumbar vertebrae. Dorsal and ventral roots enter and leave the vertebral

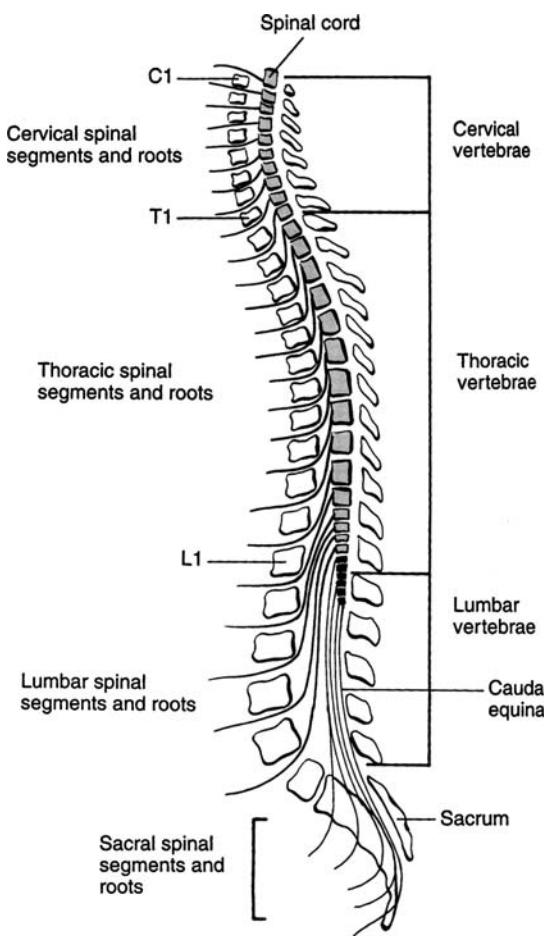


Fig. 1. The organization of the spinal cord into cervical, thoracic, lumbar, and sacral segments. Note the exit of the lumbar and sacral roots through intervertebral foramina located caudal to the spinal segment with which the root is associated.

column through intervertebral foramina at vertebral segments corresponding with the spinal segment. Because the vertebral column is longer than the spinal cord in the adult, the caudal roots are longer than the more rostral roots. At its caudal end, the cord tapers markedly to form the *conus medullaris*, and the lumbar, sacral, and coccygeal roots extending to their appropriate vertebral levels form bundles, the *cauda equina* ("horse's tail"), surrounding the *conus*.

The spinal cord is organized into 31 continuous spinal segments. These are divided into cervical (C1 through C8) segments, including those that supply the arms; thoracic (T1 through T12) segments innervating the trunk and sympathetic ganglia; lumbar (L1 through L5) segments supplying the legs, and sacral (S1 through S5) and coccygeal (one segment) segments supplying the saddle region, the buttocks, and pelvic organs, and the parasympathetic ganglia.

A *segment* is defined by dorsal roots that enter and ventral roots that exit the cord (Fig. 2). The axons in the dorsal roots arise from dorsal root ganglion cells located lateral to the vertebral column.

A pair of *dorsal root ganglia* is associated with each segment (except C1, which may have no ganglia). Each dorsal root ganglion cell gives rise to two *axonal processes*. One axonal process, the *central process*, projects through the dorsal root to terminate on neurons within the CNS. Because sensory information from the body is relayed to the CNS through the dorsal roots, axons originating from dorsal root ganglion cells are sometimes called *primary afferents*. A small population of dorsal root axons enters the spinal cord through the ventral root (ventral root afferents), but their functional significance is unclear.

The other axonal process, the *peripheral process*, enters a spinal nerve. Some of these peripheral sensory axons receive information from sensory receptors in the skin; the strip of skin supplied by the peripheral process from cells in one dorsal root ganglion is known as a *dermatome* (Fig. 3). Adjacent dermatomes overlap considerably, so that any portion of the skin is likely to be supplied by sensory axons in several peripheral nerves; damage to a single dorsal root will therefore result in little sensory loss. Other peripherally directed axons innervate sensory organs in muscles, the *muscle spindles*.

Axons from motor neurons in the spinal cord exit in the ventral roots and innervate skeletal muscles or autonomic ganglia. These ventral root axons join with the peripheral processes of the dorsal root ganglion

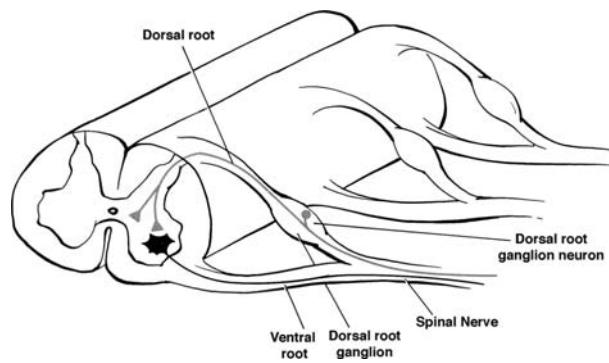


Fig. 2. In this diagram, three dorsal roots enter the dorsal lateral surface of the cord, and three ventral roots exit. The dorsal root ganglion contains dorsal root ganglion cells whose axons bifurcate; the central process enters the spinal cord in the dorsal root, and the other extends peripherally to supply the skin and muscles of the body. The ventral root is formed by axons from motor neurons exiting the spinal cord. These axons innervate muscle and autonomic ganglia.

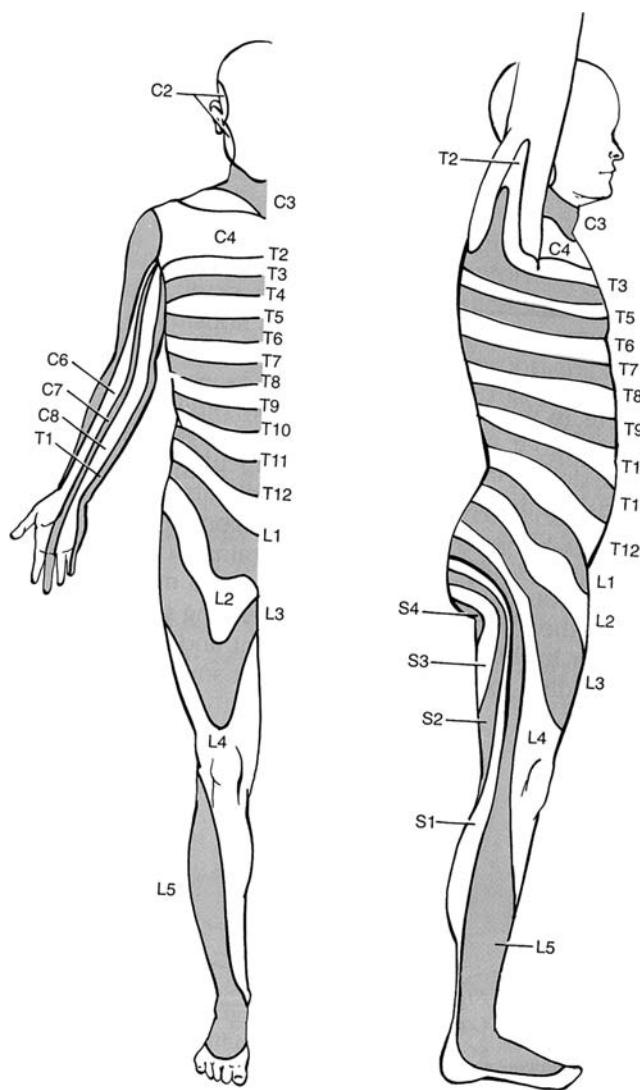


Fig. 3. A dermatome is the area of skin supplied by the peripheral processes from a single dorsal root ganglion.

cells to form spinal nerves, which thus contain both sensory and motor axons. Several spinal nerves may join to form a peripheral nerve, which is, of course, also mixed sensory and motor. The axons in the peripheral nerves are classified according to diameter, and this provides a useful correlation with the functions they serve (Table 1).

2. SPINAL NEURONS ORGANIZED INTO NUCLEI AND INTO LAMINAE

In cross-sections, the spinal cord is composed of a butterfly-shaped core of *gray matter* (cell bodies and their processes) surrounded by *white matter* (axons). Many of the axons are myelinated, which imparts the characteristic pallor to the white matter. The gray

matter is subdivided into a sensory portion, the *dorsal* (or *posterior*) horn, and a motor portion, the *ventral* (or *anterior*) horn, separated by an *intermediate zone* (Fig. 4).

The neurons in the spinal gray matter are classified according to their projections. These include *sensory relay neurons*, which receive dorsal root input and whose axons project into the ascending pathways, *motor neurons* with axons that exit in the ventral roots, and *propriospinal* cells, which are spinal interneurons whose axons synapse within the spinal cord. Propriospinal interneurons are by far the most numerous, accounting for about 90% of all spinal neurons.

As in other areas of the nervous system, many of the neurons in the gray matter are organized in functionally related clusters known as *nuclei*. These nuclei may extend the length of the spinal cord, forming columns of functionally related cells. In cross sections, the neurons in the gray matter can also be seen to have a laminated distribution, particularly in the dorsal horn. Because the histologic differences between laminae reflect functional differences, the spinal gray matter is sometimes also classified into laminae. These schemes for classifying spinal neurons are compared in Table 2.

The dorsal horn and intermediate zones (laminae I through VII) contain sensory relay nuclei, including the *marginal* (lamina I) and *proprius* (lamina III, IV) nuclei and the *substancia gelatinosa* (lamina II). Motor neurons are subdivided functionally into *somatic* and *visceral motor neurons*. Somatic motor neurons are located in the ventral horn (laminae VIII and IX) at all spinal levels and innervate striated muscle. All visceral motor neurons are located in the intermediate zone (lamina VII) at C8 through L3 (sympathetic) or S2 through S4 (parasympathetic), and innervate neurons in autonomic ganglia.

3. ASCENDING AND DESCENDING TRACTS IN WHITE MATTER

The white matter is subdivided into dorsal (ascending), lateral (ascending and descending), and ventral (descending) *funiculi*, demarcated by the dorsal medial sulcus, the dorsal root entry zone, the ventral roots, and the ventral medial sulcus (Fig. 5). The axons in the white matter form pathways that are classified as ascending (sensory), descending (motor), or propriospinal; individual pathways or tracts run in specific funiculi.

Cells in the dorsal root ganglia and spinal gray matter give rise to the axons that form the pathways

Table 1
Axons in Peripheral Nerves

Fibers	Diameters (μm)	Conduction velocity (m/s)	Role or receptors innervated
<i>Sensory</i>			
Ia (A- α)	12–20, myelinated	70–120	Muscle-spindle afferents.
Ib (A- α)	12–20, myelinated	70–120	Golgi tendon organ; touch and pressure receptors.
II (A- β)	5–14, myelinated	25–70	Secondary afferents of muscle spindle; touch, pressure, and vibratory sense receptors.
III (A- δ)	2–7, myelinated	10–30	Crude touch and pressure receptors; pain and temperature receptors; viscera.
IV (C)	0.5–1.0, unmyelinated	<2.5	Pain and temperature receptors; viscera.
<i>Motor</i>			
Alpha (A- α)	70–120, myelinated	15–20	Alpha motor neurons innervating extrafusal muscle fibers.
Gamma (A- γ)	3–6, myelinated	15–30	Gamma motor neurons innervating intrafusa neurons muscle fibers.
Preganglionic autonomic fibers (B)	<3, lightly myelinated	3–15	Autonomic ganglia.
Postganglionic autonomic postganglionic fibers (C)	0.3–1.3, unmyelinated	0.7–2.3	Viscera.

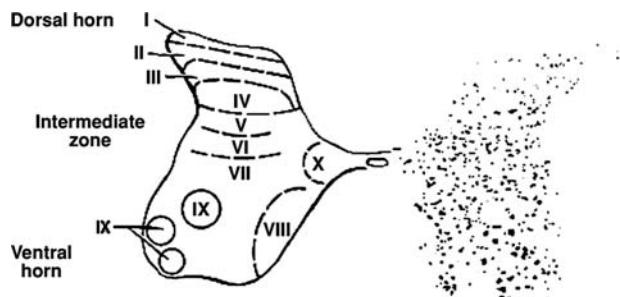


Fig. 4. Comparison of the classification schemes for the neurons in spinal cord gray matter. (Right) A section stained for neurons shows neuronal distribution and location of nuclei in the lumbar spinal cord; (left) the related laminar boundaries.

that ascend in the dorsal and lateral funiculi. Cell bodies whose axons course in descending tracts are located in many parts of the brain. Their axons descend in the lateral and ventral funiculi and terminate on motor neurons or on interneurons that project to motor neurons within the spinal gray matter. Descending systems provide central control of movement

Table 2
Classification of Spinal Neurons

Gray matter subdivision	Lamina	Nuclei included in laminae
Dorsal horn	Lamina I	Marginal nucleus
	Lamina II	Substantia gelatinosa
	Lamina III, IV	Nucleus proprius
	Lamina V	Reticular nucleus
Intermediate zone	Lamina VI	Commissural nuclei
	Lamina VII	Clarke's, intermediolateral nuclei
Ventral horn	Lamina VIII	Medial motor nuclei
	Lamina IX	Lateral motor nuclei
Commissure	Lamina X	Central gray

and posture. Some descending axons terminate on sensory-relay neurons in the dorsal horn and can therefore modify sensory input to the CNS.

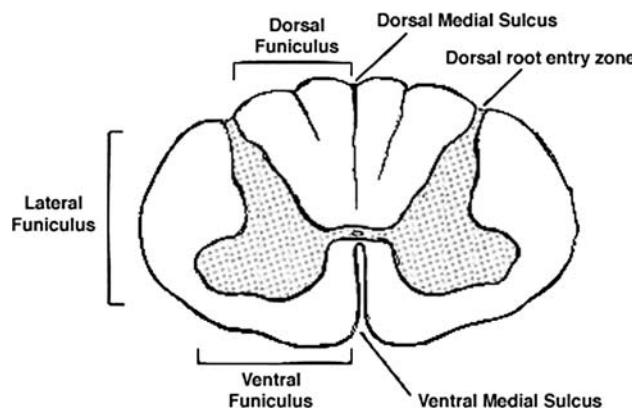


Fig. 5. Cross section of cervical spinal cord shows major landmarks and divisions of white matter.

Propriospinal pathways originate and terminate in the spinal cord itself. These axons may be very short, connecting one cell with its neighbor, others (commissural fibers) cross the midline dorsal or ventral to the central canal, and others ascend or descend in the white matter and connect distant segments of the cord (e.g., segments supplying upper and lower limbs).

3.1. Sensory Tracts Ascend

The input to the CNS from the body must be organized in such a way that information about modality (i.e., type of sensation) and location of peripheral stimulation can be used to produce appropriate spinal reflexes and to be transmitted to appropriate parts of the brain for sensory processing. Information about a painful stimulus to the skin is distributed in pathways that are different from those that transmit information about nonpainful stimuli, such as light pressure.

Two classes of dorsal root ganglion cells can be recognized (Table 1): larger cells, whose axons are myelinated (groups I and II or A α and A β fibers), and smaller cells, whose axons are unmyelinated or thinly myelinated (groups III and IV or A delta and C fibers). The small dorsal root ganglion cells are further differentiated by their synthesis of a variety of peptides (e.g., substance P, somatostatin), which are used as neuro-modulators or neurotransmitters.

At the dorsal root entry zone, the dorsal root fibers separate into a lateral and a medial division. The lateral division contains finer myelinated and unmyelinated fibers, originating from small dorsal root ganglion cells, and transmit responses to nociceptive (painful), nondiscriminatory or crude touch and thermal stimulation of the skin and viscera. The medial division contains large-caliber fibers from large

dorsal root ganglion cells, whose peripheral receptors lie in muscle, joints, and skin. These fibers relay information about muscle length and tension to interneurons and motor neurons at segmental levels, which provides the basis for spinal reflexes, and for information about somesthesia and joint position to the brain, which provides the basis for stereognosis (i.e., identification of shape and size of an object).

3.1.1. LATERAL DIVISION

Axons in the lateral division form a bundle, the tract of *Lissauer* (Fig. 6), at the dorsal root entry zone. These small-diameter axons convey the modalities of pain, temperature, and crude or nondiscriminatory touch. The axons may branch and the branches ascend or descend in Lissauer's tract for several segments before entering into the lateral portion of the dorsal horn to synapse on cells in the dorsal horn (marginal nucleus, substantia gelatinosa, nucleus proprius, laminae I to IV). This distribution of collaterals rostrally and caudally means that activation of axons in one segment of the lateral division may stimulate dorsal horn cells over several adjacent segments.

Neurons in the dorsal horn relay sensory information received from dorsal root axons to nuclei in the brain. These second-order neurons transmit this information through axons that ascend in the lateral funiculus. Sensory-relay neurons also receive input from various descending tracts, either directly or via interneurons. In this way, the cell's ability to respond to sensory input is modified by the brain.

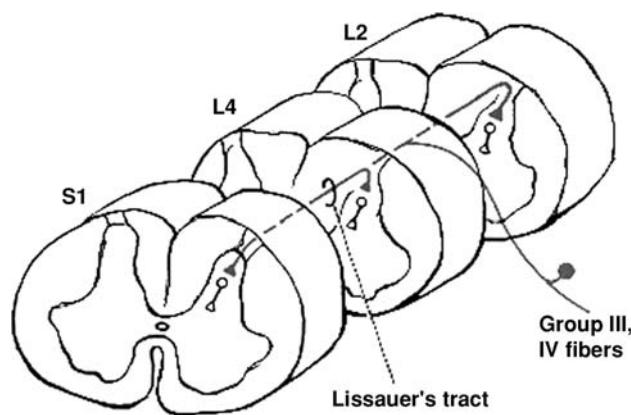


Fig. 6. Entry and central course of axons of the lateral division from one dorsal root ganglion. These unmyelinated and thinly myelinated axons may branch and ascend or descend several segments in the tract of Lissauer before entering the gray matter and synapsing on second-order neurons in the dorsal horn.

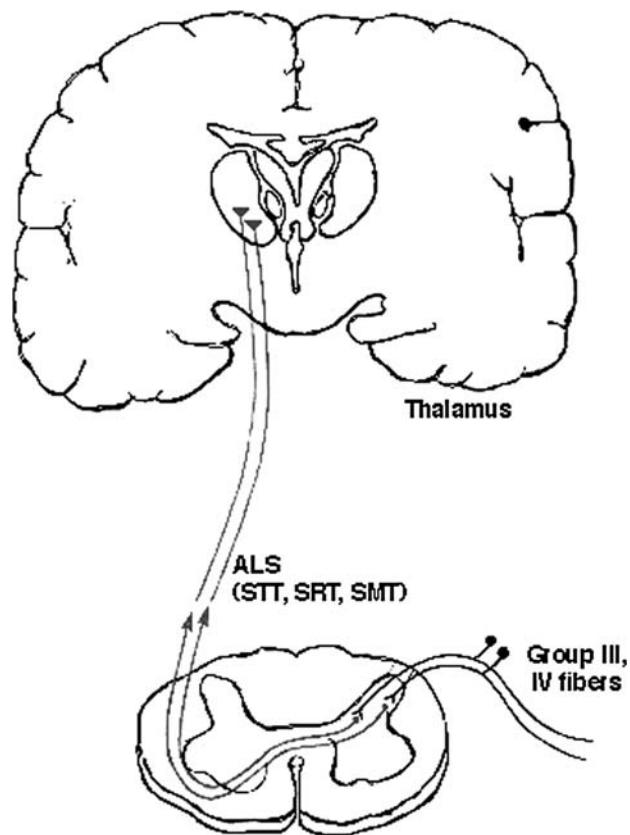


Fig. 7. Formation of the anterolateral system (ALS). Lateral division axons entering the dorsal root synapse on second-order neurons, giving rise to axons that cross the spinal cord. Some axons form the spinothalamic tract and ascend to terminate in the thalamus. Other ALS axons ascend to terminate in the reticular formation (spinoreticular tract; SRT) or mesencephalon (spinomesencephalic tract; SMT).

The location of an important ascending tract, the *spinothalamic tract* (STT), is shown in Fig. 7. Most axons in the STT arise from the marginal cells in lamina I and from the nucleus proprius in lamina III and IV and transmit nociceptive and thermal information. The axons of these second-order neurons cross to the contralateral spinal cord through the ventral commissure and ascend in the white matter as the spinothalamic tract to terminate in the thalamus and other targets in the brain. The STT, along with other pathways that ascend to the reticular formation (spinoreticular tract) and mesencephalon (spinomesencephalic and spinotectal tracts), are located in the ventral (or anterior) lateral portion of the white matter and are sometimes referred to collectively as the *anterolateral system* (ALS). The function of tracts in the anterolateral system appears to be similar to that of the spinothalamic tract, relaying pain, temperature, and crude touch sensations to the brain.

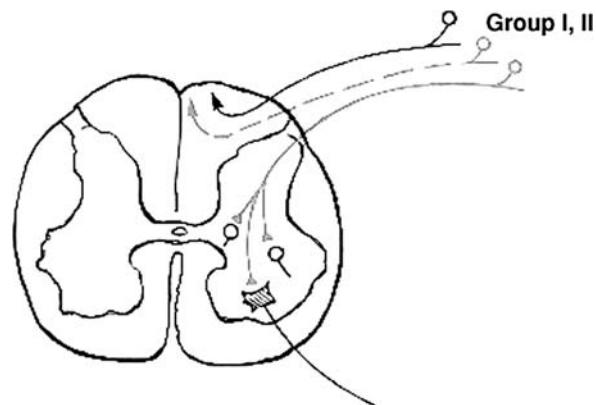


Fig. 8. Axons forming the medial division of the dorsal root enter the spinal cord and then continue into the gray matter at their level of entry, making reflex connections with motor neurons and interneurons at the level of entry, or they ascend in the dorsal columns to terminate at more rostral spinal or brain-stem levels.

Axons that contribute to the anterolateral system join the ascending pathways at each spinal segment. As the contribution from one segment enters the white matter, it displaces the fibers from lower segments in a dorsolateral direction. Therefore, these also become laminated and topographically organized. The fibers carrying sensation from the lower limbs are located dorsal and lateral to those representing the upper limbs.

3.1.2. MEDIAL DIVISION

Dorsal root axons in the medial division have local targets in their segments of entry. They may also give off collateral axonal branches that ascend to terminate in more distant targets in somatosensory relay and cerebellar relay nuclei.

Axons with local segmental targets enter the gray matter at the level of entry of the dorsal root, synapse on interneurons or motor neurons at that segmental level, and subserve reflex organization (Fig. 8). The Ia fibers of the medial division whose peripheral processes innervate stretch receptors in muscle spindles send central processes into the ventral horn, which synapse on the dendrites of motor neurons and on interneurons. The monosynaptic connection between sensory axons and motor neurons is the anatomic basis for an important reflex, the *stretch reflex*.

Other medial division axons, including collaterals of axons with segmental targets, enter the white matter in the dorsal funiculus, where they ascend to terminate in relay nuclei for somatosensory or cerebellar pathways. These relay nuclei receive sensory information from the skin, muscles, joints, fascia, and other

tissues, then transmit the information to nuclei in the thalamus. The thalamus relays information to the cortex, where it is consciously appreciated or to the cerebellum, where it contributes to the control of posture and movement, without being consciously perceived.

Dorsal root fibers that project to somatosensory relay nuclei enter the dorsal funiculus at each segment, medially displacing the fibers originating from more caudal ganglia. As a result, fibers become laminated (topographically organized). In the cervical region, fibers from sacral dorsal roots are found nearest the midline and those from cervical roots nearest the dorsal root entry zone. Fibers representing the lower half of the body (sacral to T5) ascend in the *gracile fasciculus*; those from the upper half (T5 to C2) comprise the *cuneate fasciculus*. Axons in both bundles terminate ipsilaterally in nuclei of the medulla for which they are named, the nucleus gracilis and cuneatus. The gracilis and cuneate nuclei then relay impulses to the thalamus (Fig. 9).

Other dorsal root axons enter the dorsal funiculus to ascend for several segments before terminating in relay nuclei for cerebellar pathways. Axons arising

from dorsal root ganglion cells in caudal thoracic, lumbar, and sacral regions ascend in the dorsal columns to terminate in *Clarke's nucleus* (Fig. 10), located in segments T1 to L2. Those arising from more rostral ganglia ascend in the dorsal columns to terminate in the *lateral* (or *external* or *accessory*) *cuneate nucleus* in the medulla.

The *dorsal spinocerebellar tract* (DSCT) arises from neurons located in Clarke's nucleus; it ascends in the white matter ipsilaterally and is therefore uncrossed (Fig. 10). The DSCT terminates in the cerebellum. Similarly, the *cuneocerebellar tract* carries information from the lateral cuneate nucleus to the cerebellum. Both nuclei therefore relay sensory information from the periphery to the cerebellum. There is a third pathway to the cerebellum, the ventral spinocerebellar tract (VSCT). Cell bodies whose axons form the VSCT are distributed throughout the dorsal horn and intermediate zone; their axons cross in the ventral commissure to ascend in the contralateral VSCT to the cerebellum, where they cross again before terminating. Although the course of the VSCT differs from that of the DSCT and cuneocerebellar pathways, their functions appear to be similar.

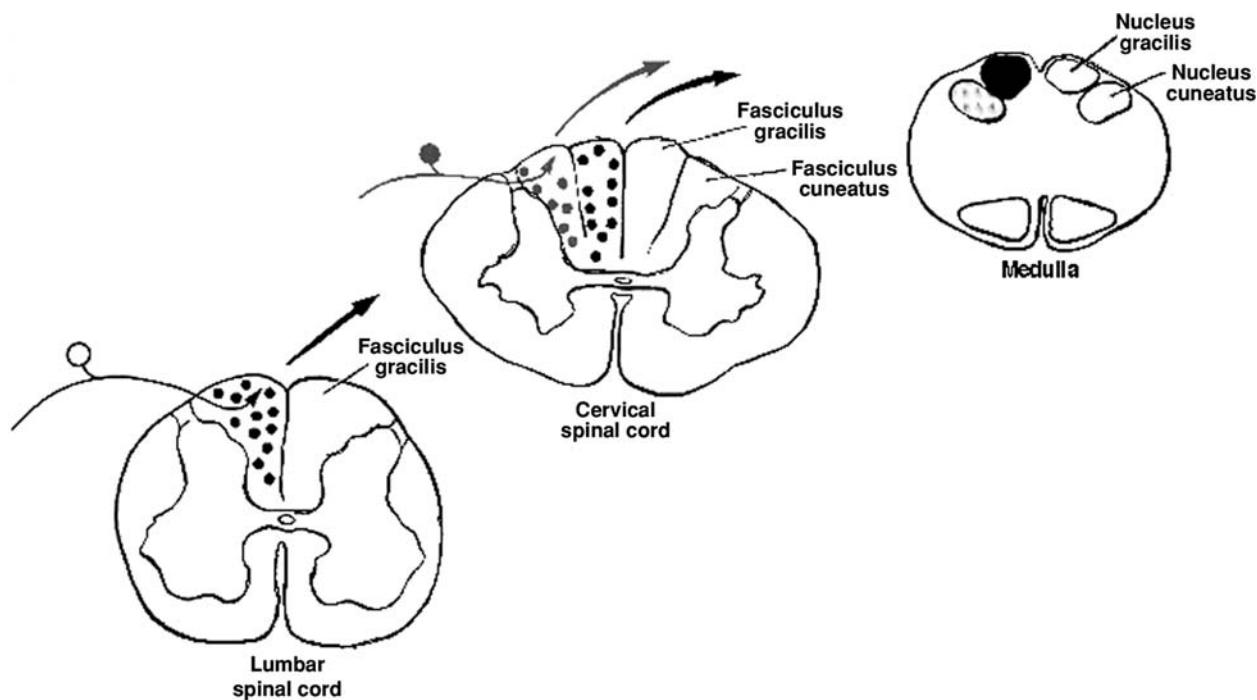


Fig. 9. Axons that mediate fine tactile sensibility form part of the medial division. They ascend in the dorsal columns to the brain stem, where they terminate on second-order neurons in the dorsal column nuclei. The axons arising from lumbar and low thoracic dorsal root ganglia ascend in the fasciculus gracilis and terminate in the nucleus gracilis. Axons arising from upper thoracic and cervical ganglia ascend in the more laterally located fasciculus cuneatus and terminate in the nucleus cuneatus located lateral to the nucleus gracilis in the medulla.

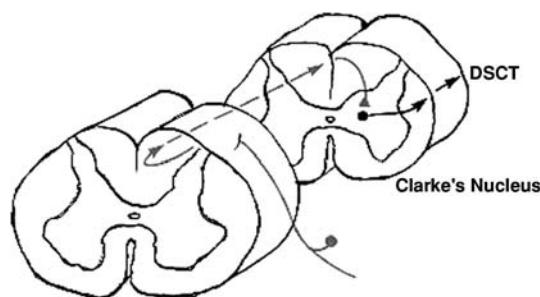


Fig. 10. Axons conveying proprioceptive and muscle information form part of the medial division. Axons from lumbar and caudal thoracic dorsal root ganglia enter the spinal cord and ascend in the dorsal columns to the thoracic level, where they terminate on second-order neurons in Clarke's nucleus. The axons of Clarke's neurons ascend in the lateral funiculus as the dorsal spinocerebellar tract (DSCT), which terminates on third-order neurons in the cerebellum.

3.2. Central Motor Pathways Descend

The axons that form the central motor pathways arise from cell bodies located at all levels of the brain stem and the cerebral cortex. These cells are often called *upper motor neurons* to distinguish them from motor neurons in the spinal cord (*lower motor neurons*) that innervate muscle directly. Many of these descending motor tracts undergo partial or complete decussation (crossing) before entering the cord. However, some descend ipsilaterally, enter the gray matter, and then cross in the spinal commissure to terminate on neurons contralateral to their origin. Still other tracts are primarily ipsilateral.

The descending tracts are located in the lateral and ventral funiculi. Most of the axons in these tracts

terminate on interneurons, which then project to motor neurons; very few terminate directly on motor neurons. Some descending axons terminate on sensory relay neurons, which provides central control over sensory processing. The location of four important descending tracts, the *corticospinal*, *rubrospinal*, *reticulospinal*, and *vestibulospinal*, tracts, is shown in Fig. 11.

The *corticospinal tract* arises from the motor, pre-motor, and somatosensory cortex, descends to the spino-medullary junction, where 90% of the axons cross, and then continues to descend as the lateral corticospinal tract in the lateral funiculus contralateral to the cell bodies of origin. Those axons that do not cross at the spino-medullary junction descend in the spinal cord as the ventral corticospinal tract but then cross in the spinal cord before their termination contralateral to their origin. The *rubrospinal tract* arises from neurons in the red nucleus; it is also virtually completely crossed. The *reticulospinal tract* contains axons that arise from reticular nuclei ipsilaterally and contralaterally. Reticulospinal pathways include axons that indirectly modulate activity of the spinal cord (e.g., serotonergic and noradrenergic axons). The *vestibulospinal tract* arises from the vestibular nuclei in the brain stem. Most of the axons in the vestibulospinal tract are uncrossed.

4. MOTOR NEURONS

4.1. Topographic Organization of Somatic Motor Neurons

Somatic motor neurons in the ventral horn of the spinal cord express the activity of the CNS. Sherrington

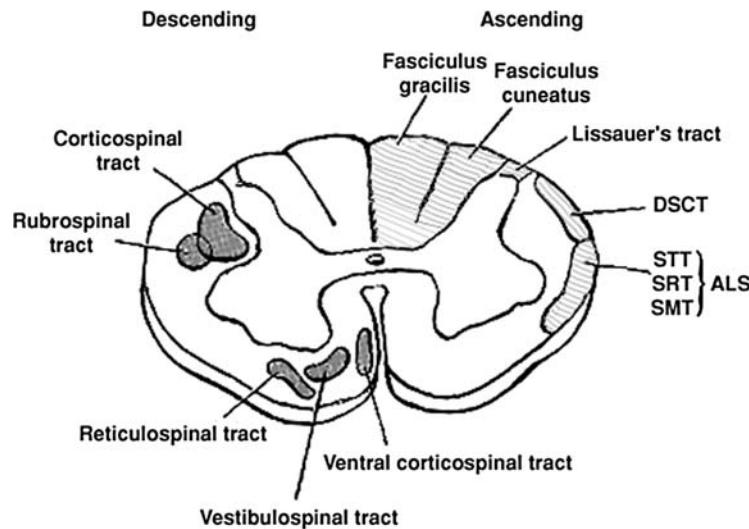


Fig. 11. The approximate location of the ascending tracts is shown on the right and of the descending tracts on the left. DSCT: Dorsal spinal cerebellar tract; STT: spinothalamic tract; SMT: spinomesencephalic tract; SRT: spinoreticular tract; ALS: anterolateral system

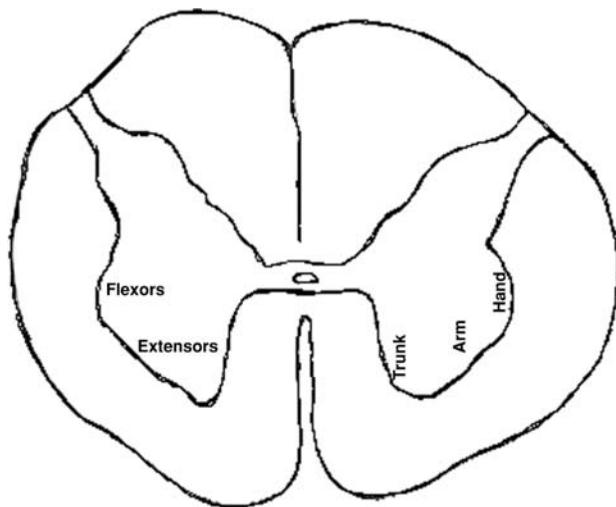


Fig. 12. Somatic motor neurons are organized topographically in the ventral horn. Axial musculature is supplied by motor neurons located medially and limb musculature is supplied by motor neurons located laterally in the ventral horn. Flexor motor neurons are dorsal and lateral to motor neurons innervating extensor muscles.

called them the “final common pathway.” Axons from somatic motor neurons exit in the ventral roots and innervate striated muscle. These neurons are located exclusively in the ventral horn (laminae VIII and IX). A medial motor nucleus is present in the ventral horn throughout the length of the cord. These motor neurons innervate axial (trunk) musculature. There are also prominent groups of nuclei located laterally in the ventral horn, which are particularly well developed in the segments supplying the limbs. The lateral group of nuclei is subdivided functionally into ventral nuclei, which innervate extensor muscles, and dorsal nuclei, which innervate flexors. Within these two subdivisions, the nuclei innervating proximal muscles are located medially and those that supply the distal muscles more laterally (Fig. 12).

Motor neurons are among the largest in the spinal cord. Although the motor neuron cell bodies are localized into discrete nuclei, their dendrites extend into the intermediate zone, the dorsal horn, and even into the white matter. This allows considerable convergence of input onto a motor neuron, and in fact a single motor neuron may receive as many as 10,000 axon terminals from many different sources.

All motor neurons use the excitatory transmitter acetylcholine. The axons of many motor neurons give off a collateral before exiting in the ventral root. These short *recurrent collaterals* terminate on interneurons. Some of these interneurons are part of a disynaptic pathway that inhibits motor neurons (motor axon

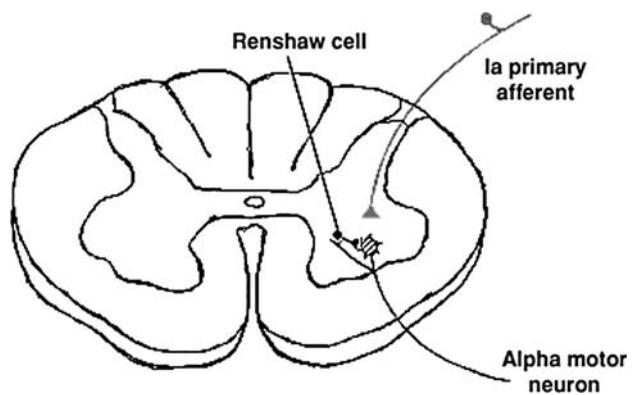


Fig. 13. Renshaw cells. A Ia primary afferent axon makes a monosynaptic contact with an alpha motor neuron whose axon innervates a somatic muscle. The motor neuron also emits a collateral that synapses on an interneuron, the Renshaw cell, situated near the alpha motor neuron. The Renshaw cell inhibits the motor neuron (recurrent inhibition).

collateral → interneuron → motor neuron). This interneuron is called the *Renshaw cell*, and it uses the inhibitory transmitter glycine to inhibit the postsynaptic motor neuron, a phenomenon known as *recurrent inhibition*. This feedback circuit permits the regulation of activity in motor neurons by the motor neurons themselves, acting to focus activity by limiting the duration of the activation (Fig. 13).

4.2. Visceral Motor Neurons in the Intermediate Zone

Visceral motor neurons innervate neurons in autonomic ganglia and are also called preganglionic neurons. The autonomic ganglion cells (postganglionic neurons) innervate visceral organs. Those preganglionic neurons in the *intermediolateral nucleus* in lamina VII at C8 to L3 send axons to the ganglia in the sympathetic chain and provide central regulation of the sympathetic nervous system. Neurons in the intermediate zone at levels at S2 through S4 form the more poorly defined *sacral parasympathetic nucleus*. Their axons innervate the sacral parasympathetic ganglia and thus provide central control of the sacral portion of the parasympathetic system that innervates the bowel, bladder, and sexual reflexes. Central control of more rostral portions of the parasympathetic system is provided by groups of cranial nerve nuclei.

5. REGIONAL SPECIALIZATIONS

At all segmental levels of the spinal cord, the dorsal horn (laminae I through IV), the intermediate zone (laminae V through VII), and the ventral horn

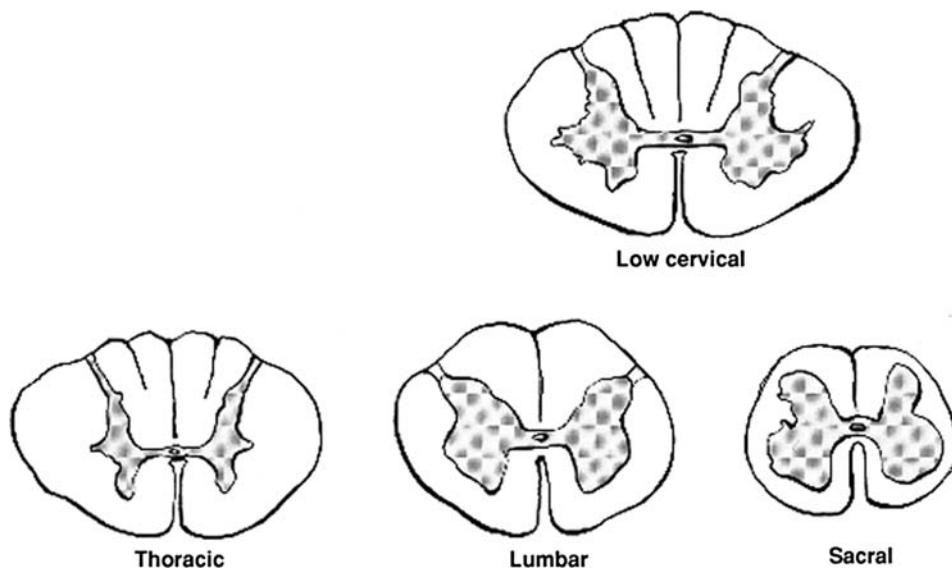


Fig. 14. The configuration of gray and white matter at various levels of the spinal cord differs in characteristic ways.

(laminae VIII and IX) can be recognized. Regional specializations modify the butterfly shape of the gray matter in characteristic ways. In the spinal segments that innervate the limbs (the cervical and lumbar enlargements), the number of neurons is greatly increased and the dorsal and ventral horns are concomitantly expanded. Two nuclei located in the intermediate zone, Clarke's and the intermediolateral nuclei, are prominent only in segments that do not innervate the limbs (T1 through L2). Therefore, the gray matter has different silhouettes that are characteristic of the particular spinal levels (cervical, thoracic, lumbar, or sacral) (Fig. 14).

The amount of white matter also differs according to segment. Ascending pathways become larger more rostrally because axons from dorsal root ganglia or from sensory-relay nuclei are added at each segment. Most descending tracts send fibers to terminate in gray matter at all segments. Descending tracts are thus smaller at more caudal levels, because they are continuously depleted of fibers. The circumference of the caudal spinal cord is therefore also smaller than at rostral levels, because there is less white matter caudally.

6. SPINAL REFLEXES

The activity of dorsal root axons is expressed, monosynaptically or polysynaptically, on motor neurons whose axons form the ventral root. This arrangement comprises the *segmental organization* of the spinal cord, and it is this organization that

determines the reflex activity of the spinal cord (i.e., the reflex activity that persists after a spinal transection that separates the brain from the spinal cord). *Spinal reflexes* are stereotyped responses (contraction, relaxation) made by somatic muscles in response to stimuli that excite receptors in muscle, tendon, or skin. These reflexes are more readily demonstrable in a spinal transected preparation than in an organism with an intact spinal cord, as the reflex pathways are normally subjected to descending inhibition, but these reflexes make a major functional contribution in providing muscle tone, posture, and enabling voluntary movement for the organism.

6.1. The Stretch Reflex Determines Muscle Tone

When a muscle is passively stretched, it responds by contracting. This is a result of the stimulation of receptors located in sensory organs, the muscle spindles. This stretch (or *myotatic* or monosynaptic) reflex provides the basis for muscle tone—the slight resistance to stretch found in all healthy innervated muscles. Testing this reflex allows the examiner to evaluate the excitability of motor neuron pools.

A striated muscle is composed of two types of fibers: a large number of *extrafusal muscle fibers* and a smaller number of highly specialized sensory structures, the *muscle spindles* (Fig. 15). The muscle spindle is a fusiform structure containing several modified muscle fibers, the *intrafusal* fibers, and the axons that innervate them. Each spindle is encased in a connective tissue capsule.

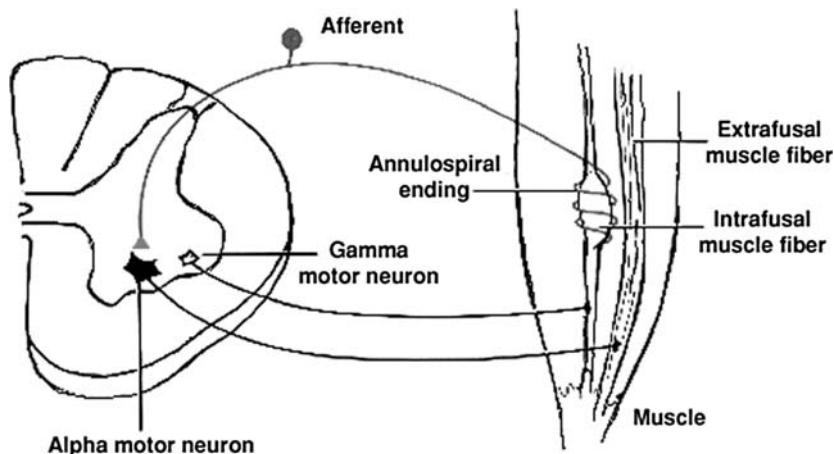


Fig. 15. The gamma loop. A small gamma motor neuron, located near an alpha motor neuron, innervates the poles of intrafusal fibers. The alpha motor neuron innervates extrafusal muscle fibers in the same muscle. Activity in the gamma motor neuron causes contraction of the poles of the intrafusal fiber, which stretches the central zone and activates the peripheral process of the Ia afferent. The Ia afferent is shown innervating the intrafusal fiber peripherally and the alpha motor neuron centrally (the monosynaptic reflex).

There are also two types of somatic motor neurons: *alpha* and *gamma motor neurons*. Alpha motor neurons are large neurons that innervate the extrafusal muscle fibers, the major contractile element of the muscle. They are grouped into nuclei that innervate individual muscles. These nuclei also contain small *gamma motor neurons*, which innervate the intrafusal muscle fibers and control the sensitivity of the muscle spindle to stretch. Gamma motor neurons are located near the alpha motor neurons that innervate extrafusal fibers of the same muscle.

The central portion of the intrafusal fiber is non-contractile; this zone is innervated by Ia afferent fibers, which make “annulospiral” endings around the noncontractile central zone. The intrafusal fibers are also innervated by group II afferents, which make another type of ending, the “flower spray” endings, upon them. Only the polar regions of the intrafusal fiber are contractile. Each pole receives innervation from gamma motor neurons. The polar regions of intrafusal fibers respond to gamma efferent stimulation with a slow, maintained (tonic) contraction, thereby stretching the intrafusal fiber that in turn stretches the central region, thus stimulating the Ia and/or II afferent axons.

The central processes of the Ia and II fibers terminate on cells in the spinal gray matter, including the alpha motor neurons innervating the muscle that the peripheral process of the Ia fiber contacts (Fig. 16A). There are also Ia terminals on motor neurons that innervate synergists. Other important terminations of the Ia fiber are on interneurons, some of which inhibit

the motor neurons that innervate muscles that act antagonistically to that of the Ia fiber. There is one synapse in the excitatory path (monosynaptic pathway) and two synapses (disynaptic pathway) or more involved in the inhibitory pathways (Fig. 16B). Passive stretch of a muscle will therefore facilitate contraction of that muscle and its synergists, and, with a slight delay, inhibit contraction of the antagonist muscles.

6.2. The Inverse Myotatic Reflex Limits the Stretch Reflex

When a muscle contracts, another type of muscle receptor, the *Golgi tendon organ*, is stimulated. These receptors are located in tendons close to their junctions with muscle and are stretched during muscle contraction. Golgi tendon organs therefore measure muscle tension. They receive sensory innervation from Ib dorsal root axons, but, unlike muscle spindles, they do not receive motor innervation. The central process of the Ib fiber terminates on interneurons that inhibit motor neurons of the muscle of origin (e.g., homonymous muscles) and facilitate the antagonists (e.g., heteronymous muscles). This reflex pathway limits (applies “brakes” to) the muscle contractions (Fig. 17).

6.3. The Flexor and Crossed-Extensor Reflex Constitute Another Protective Mechanism

Noxious or thermal stimulation of the skin or deep tissues excites group III and IV axons in the

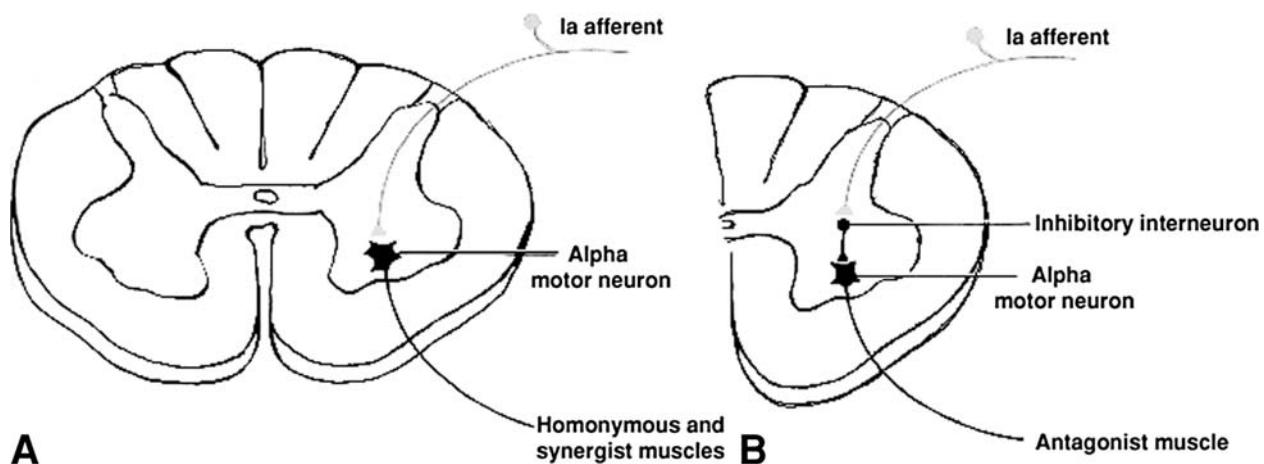


Fig. 16. (A) Monosynaptic reflex pathway. Ia afferents supplying muscle spindles in somatic muscles make monosynaptic excitatory contacts on alpha motor neurons supplying the same muscle and synergistic muscles. Stretch of the muscle produces contraction of that muscle and its agonists. (B) Disynaptic inhibitory reflex pathway. Ia afferents also make excitatory contacts on inhibitory interneurons that make synaptic contact with alpha motor neurons that supply muscles that are antagonists to the muscle supplied by the Ia afferent. Stretch of the muscle also produces relaxation of opposing muscles.

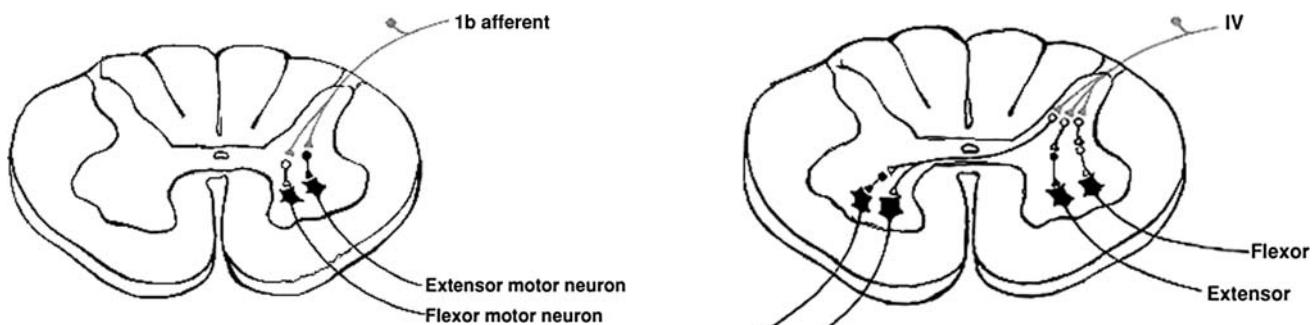


Fig. 17. The inverse myotatic reflex. The peripheral process of a Ib afferent innervates a Golgi tendon organ from an extensor muscle; the central process makes excitatory contacts with two interneurons. The excitatory interneuron synapses on motor neurons supplying flexor muscles, and the inhibitory interneuron synapses on motor neurons supplying extensor motor neurons. Continued stretch of the muscle thus produces relaxation.

peripheral nerves. This information is transmitted to motor neurons on both sides of the cord through interneurons. These polysynaptic pathways permit a divergence of the sensory stimulation so that neurons in many ipsilateral segments and the contralateral side of the cord may be recruited. This type of stimulation produces ipsilateral excitation of flexors and inhibition of extensors and contralateral inhibition of flexors and excitation of extensors. This reflex pattern is known as the *flexor and crossed-extensor* reflex (Fig. 18).

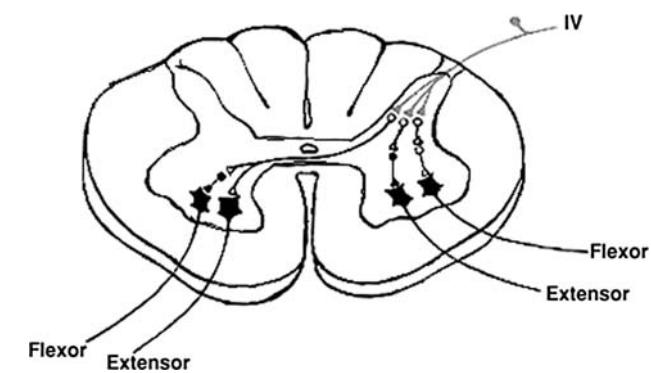


Fig. 18. The flexor-crossed extensor reflex. Painful stimulation activates small-diameter axons that make contact with interneurons within the gray matter. Through polysynaptic pathways, using interneurons that inhibit and excite flexor and extensor motor neurons on both sides of the spinal cord, the ipsilateral limb flexes—a protective response—and the contralateral limb extends, providing postural support.

6.4. Central Pattern Generators

Not all movement is based on simple reflexes. Within the spinal gray matter there are networks of interneurons and motor neurons distributed on each side of the spinal cord (half-centers) and interconnected through commissural pathways. These networks form the spinal *central pattern generators* (CPG). The CPG activates motor neurons to produce the rhythmic alternating patterns of flexion and extension that comprise the stepping patterns that underlie locomotion. The CPG is normally influenced by both

primary afferents and descending pathways but can provide rhythmic activity to motor neurons even when isolated. Thus the basic machinery for locomotion, including the capacity to generate ordering of muscle activation and coordination among limbs and their reflex responses, is intrinsic to the spinal cord. In humans, the CPG can be most readily revealed in infants and after electrical epidural stimulation in paraplegics, individuals suggesting a level of descending inhibition in the adult.

7. SPINAL LESIONS

7.1. Spinal Transection Interrupts Neuronal Transmission

Transection of the spinal cord interrupts descending input from the brain to spinal levels below the level of transection and input to the brain ascending from sensory structures located below the level of transection. A patient whose spinal cord is transected will undergo a period of “spinal shock,” characterized by flaccid paralysis of muscles innervated by motor neurons below the transection. Voluntary control of muscles innervated by motor neurons and perception of sensory events arising below the level of the lesion is permanently lost. Over time, the flaccid paralysis is followed by a bilateral, symmetric spasticity of muscles below the level of the lesion. This hyperactivity after recovery from spinal shock is caused by the loss of descending inhibitory influences on spinal neurons.

7.2. Spinal Hemisection Generates Both Ipsilateral and Contralateral Impairment

Hemisection usually results from a slow-growing mass in the spinal cord that deforms the cord, compressing descending and ascending axons on one side of the cord and producing functional disorders. Important descending pathways cross at supraspinal levels so that the motor impairments are ipsilateral and below the level of the hemisection. Second-order sensory fibers in the dorsal columns ascend ipsilaterally, and thus some sensory impairments are also ipsilateral and below the level of the hemisection. Other sensory pathways (e.g., spinothalamic tract) cross in the spinal cord and ascend contralaterally, and the impairments associated with these pathways are expressed contralateral to the lesion. Thus motor function and discriminatory tactile and kinesthetic sense are lost ipsilaterally at levels below the lesion, and pain and thermal sensitivity are lost contralaterally below the lesion. A neurologic examination

reveals spastic paralysis and diminished touch sensation on the side of the body that is ipsilateral to the lesion, plus a loss of pain and temperature sensation on the side contralateral to the lesion. The pattern of symptoms resulting from a hemisection is known as the Brown-Séquard syndrome.

7.3. Syringomyelia Produces Bilateral Impairment

Syringomyelia refers to a pathologic enlargement (cavitation) of the central canal. As the cavity expands dorsally and ventrally, it first interrupts the fibers that cross through the anterior and ventral commissures in the spinal cord, including some ascending sensory pathways. With further enlargement of the cavity, wasting and motor dysfunction may occur, particularly in axial muscles innervated by medial motor neurons, indicating encroachment on the ventral horn. In the cervical spinal cord, where syringomyelia is prone to occur, interruption of decussating sensory pathways results in bilateral loss of pain and temperature sensation, but with preservation of tactile and kinesthetic sensation.

7.4. Pathologic Reflexes Have Several Causes

Spasticity is an abnormal increase in muscle tone associated with a loss of descending inhibition of gamma and alpha motor neurons. *Rigidity* is an abnormal increase in muscle tone caused by loss of inhibition of alpha motor neurons. *Flaccidity* is an absence of muscle tone that occurs after loss of peripheral nerve innervation.

7.4.1. SPASTICITY

Spasticity is a common and severe consequence of spinal injuries in which descending regulation of spinal circuits is impaired. Passive stretch of a muscle evokes a reflex contraction of that muscle. The strength of this monosynaptic stretch reflex is controlled by the gamma motor neurons that innervate that muscle. These gamma motor neurons, in turn, are under excitatory and inhibitory drive from the CNS. Normally, there is a balance of excitation and inhibition, which is reflected in some tension in the polar regions of the intrafusal fibers. This tension stimulates Ia fibers, producing a basal level of excitation of the alpha motor neurons. This balance produces muscle tension or “tone.” Increased excitation or decreased inhibition of gamma motor neurons increases tension on the intrafusal fibers, which then become hypersensitive to stretch of the whole muscle, resulting in

an abnormally increased contraction in response to stretch, the phenomenon known as *spasticity*.

Under such conditions, changes in reflexes are clearly observable clinically. If the stretch is applied continuously to a spastic muscle, the resistance increases but then suddenly gives way, as a result of inhibition by the Golgi tendon organ system. This sudden collapse of resistance is known as the lengthening reaction or clasp-knife effect. The clasp-knife reaction is only revealed when muscle tone is abnormally increased, but its basis, the activation of the Golgi tendon organ, normally contributes to muscle tone.

Spasticity is seen in muscle groups innervated by motor neurons below the level of a spinal transection. An attempt to elicit a tendon reflex produces a greatly increased response. In addition, a mild stimulus provided by stroking the lateral border of the foot will evoke the classic extensor-plantar (e.g., Babinski) response, in which the toes spread and extend instead of flexing, as they would normally do. In the adult, the Babinski response is normally inhibited by descending projections; it can be elicited in infants before the descending projections mature or in individuals with injury to these systems.

The exact etiology of spasticity is controversial. A hyperactive stretch reflex has been believed to contribute to spasticity. However, clinical studies show that not all spastic muscles exhibit an increased stretch reflex. The hyperactivity of alpha motor neurons and interneurons, as well as changes in muscle properties resulting from such lesions, undoubtedly also contribute to spasticity.

7.4.2. RIGIDITY

Rigidity is an increased activation of the alpha motor neurons, seen after damage to some descending pathways. Rigidity involves an increased resistance to movements in all directions and does not depend on the dorsal root innervation of muscle spindles. This resistance to passive motion felt when examining the patients has been likened to the feeling of bending a lead pipe (lead pipe rigidity). Occasionally, the resistance has a phasic quality, a phenomenon known as cogwheel rigidity. Parkinson's disease, which results from degeneration of neurons in the basal ganglia that indirectly project to the spinal cord, produces the purest form of clinical rigidity.

7.4.3. FLACCIDITY

A lesion to motor neurons or their axons produces a flaccid paralysis of the muscle innervated by those

neurons. No voluntary movement is possible, there is no resistance to passive movement, and no reflexes can be elicited. If axonal regeneration does not occur, the muscle will atrophy.

8. STRATEGIES TO REPAIR INJURED SPINAL CORD

Injury to the spinal cord produces severe deficits because neurons that are lost are not replaced, and axons that are cut do not regenerate. Severe injuries, such as spinal transection, eliminate supraspinal influences and thus eliminate voluntary control of movement. In recent years, several strategies have been developed in animal models with the goal of rescuing injured neurons, promoting regeneration, and restoring motor control.

The site of injury is toxic as a result of release of such substances as glutamate from dying cells or proinflammatory cytokines from cells of the immune system that migrate to the injury site. Thus neurons near the site of injury die as an indirect result of the lesion, leading to an enlargement of the injury site (secondary degeneration) and development of additional deficits. Other neurons whose axons have been severed by the lesion may atrophy or die (retrograde degeneration; an apoptotic process). Strategies aimed at countering the toxic environment with anti-inflammatory agents or blocking the apoptotic process with provision of trophic factors or antiapoptotic molecules have been successful in animal models in rescuing neurons destined to die or to atrophy both at the lesion site and distant from it.

Regeneration of CNS axons, long believed to be impossible, is now known to occur in the presence of a permissive environment. The CNS environment itself is not permissive for growth of axons. For example, the myelin sheath bears molecules that block axonal growth. The injured spinal cord is even less permissive as the scar that forms around a lesion contains molecules that also block axonal growth. Agents are being developed to counter these inhibitory compounds, and they have been shown to encourage growth of severed spinal axons in laboratory experiments. The environment can also be made more permissive by application of appropriate trophic factors. Provision of trophic factors, either directly by pumps or indirectly by transplantation of cells that produce these factors, has been successful in promoting regeneration in animal models.

Physical rehabilitation protocols have also shown promise in improving function after spinal injury. Appropriate exercise can prevent or diminish muscle atrophy after spinal cord injury. Exercise also increases the levels of trophic factors in muscle and in spinal cord, which can promote neuroprotection and axonal regeneration. Finally, physical exercise will activate the CPG and may modify the firing patterns of the generator, in a form of spinal learning, and thus has the potential to improve function.

Experimental studies have shown that some of the serious consequences of spinal injury can be ameliorated or reversed. The complexity of spinal and supraspinal circuitry will undoubtedly require a combined approach, using several therapeutic interventions to affect the repair of spinal injuries in humans.

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Disorders of the Spinal Cord

Gregory Cooper, Gerald Eichhorn and Robert Rodnitzky

Clinical abnormalities of the spinal cord lead to great disability resulting from severe disruption of motor, sensory, and autonomic functions. The distinct longitudinal organization of ascending and descending fiber tracts in the spinal cord, coupled with the segmental groupings of neurons with motor, sensory, and autonomic functions, often allow relatively precise localization of the lesion responsible for a spinal cord syndrome.

CAUSES OF SPINAL CORD DISORDERS

Clinical spinal cord syndromes are more often the result of structural damage rather than metabolic dysfunction. Although relatively minor aberrations of the metabolic milieu of the brain can result in severe neurologic dysfunction, clinical symptoms related to spinal cord dysfunction are much less likely to be of metabolic origin. Neurologic symptoms can result from spinal cord ischemia, but not as readily as is the case in cerebral ischemia. Spinal cord ischemia severe enough to produce symptoms is commonly the result of atherosclerotic occlusion of the aorta or its branches, embolization to these blood vessels, or vascular malformations within the substance of the spinal cord.

Most clinical spinal cord syndromes result from mechanical disruption or physical degeneration of the constituent cells and fiber tracts of the cord. Typical examples of conditions resulting in mechanical disruption of spinal cord elements include spinal trauma (e.g., spinal column fracture, bullet wound), spinal cord tumors, and intervertebral disk herniation causing spinal cord compression. Degenerative processes causing spinal cord symptoms include demyelination of long tracts (e.g., multiple sclerosis) and neuronal degeneration (e.g., motor neuron disease).

LOCALIZATION OF THE CAUSATIVE LESION

Precise localization of the causative lesion is extremely important in treating spinal cord disorders. Even in the era of modern neuroimaging, a thorough neurologic examination is essential in localizing the abnormality within the spinal cord. Although magnetic resonance imaging or computed tomography of the spinal cord can be performed in most communities, it is critical to know which part of the spinal cord should be imaged, and it is essential to understand whether the pathology demonstrated by the procedure can explain the patient's symptoms.

The most important clinical finding in attempting to localize spinal cord pathology is the sensory level. A sensory level is a horizontal demarcation of sensory loss on the trunk or a diagonal linear demarcation on an extremity that corresponds with the segmental innervation of skin by the paired spinal nerves leaving the spinal cord. Classically, sensation is lost below the level of a spinal cord lesion and is intact above it because of the interruption of ascending impulses in sensory spinal pathways such as the spinothalamic tracts or dorsal column pathways by the offending lesion. If the lesion involves the entire spinal cord at a given level, sensation is lost equally on both sides of the body below that level. A sensory level to pain or touch sensation is demonstrated by applying the appropriate stimulus to the skin below the level of the suspected lesion and gradually ascending up the trunk until the stimulus is felt. A sensory level of vibratory perception can be similarly established by applying a vibrating tuning fork at bony landmarks beginning with the toes, proceeding to the ankles, knees, and hips, and ultimately moving up the spinous processes of the vertebrae until a normal sense of vibration is perceived.

SPARING OF SACRAL SENSATION MAY HELP DIFFERENTIATE A LESION IN THE CENTER OF THE SPINAL CORD FROM ONE ON ITS CIRCUMFERENCE

After the fibers that transmit pain and temperature sensations enter the spinal cord, they cross the midline and ascend on the opposite side, where they are progressively displaced laterally by incoming fibers entering and crossing at higher levels. The fibers entering at the lowest (e.g., sacral) level, which are those that are the first to enter, cross, and ascend, come to occupy the most lateral position in the lumbar, thoracic, and cervical spinal cord as ascending fibers enter the spinothalamic tracts at higher levels. The extreme lateral position of fibers transmitting sacral sensation protects them from lesions deep within the center of the spinal cord, such as a midline tumor. In such cases, there may be profound loss of sensation below the level of the lesion, with the exception of the saddle or perianal area, subserved by sacral fibers, where sensation is spared. This can be extremely useful to the clinician in determining whether spinal cord pathology is intrinsic to the spinal cord or is causing symptoms by compression from outside.

In cases of external compression, the most laterally placed fibers (those subserving sacral sensation) may be involved first, and the initial sensory level may be considerably below the level of the lesion early in the illness and ascend to the true level with the passage of time as more fibers are compressed. Another clue to the possibility that a lesion may be compressing the spinal cord from the outside is the presence of radicular pain. This is a sharp, stabbing pain that follows the cutaneous distribution of a single dorsal spinal root, caused by irritation of the nerve as it enters the spinal cord. This type of pain is even more likely to occur when the offending lesion is attached to the spinal nerve, as is the case with nerve-sheath tumors. Such tumors are rare, and herniated lumbar or cervical intervertebral disks are by far the most common cause of irritation of a spinal root with resultant radicular pain.

LESIONS INVOLVING HALF THE SPINAL CORD PRODUCE A DISTINCT CLINICAL SYNDROME

The Brown-Séquard syndrome results from interruption of motor and sensory tracts on only one side of the spinal cord. In this condition, the functions of

uncrossed fibers are lost on the side of the body ipsilateral to the lesion, and the functions of crossed fibers are lost on the contralateral side. Pain and temperature sensation are lost on the side opposite the lesion because of the interruption of the crossed lateral spinothalamic tract, and weakness and loss of joint position and vibratory sensation appear on the same side because of interruption of the corticospinal tract and dorsal-column fibers, respectively, which are uncrossed in the spinal cord. Because fibers transmitting pain and temperature sensations may ascend one or two spinal cord segments on the side of entry before crossing the midline to join the contralateral spinothalamic tract, there may be loss of pain and temperature sensation ipsilateral to the side of the lesion over an expanse of skin that corresponds with one or two dermatomes at the level of the offending lesion.

A CAPE-LIKE SENSORY LOSS MAY ALSO BE A CLUE TO THE LOCALIZATION OF A SPINAL-CORD LESION

Small lesions involving the region immediately adjacent to the central canal of the spinal cord may involve the anterior white commissure. Because this structure contains fibers that convey pain and temperature sensation from both sides as they cross to join the contralateral spinothalamic tract, these modalities may be preferentially lost in the corresponding dermatomal segments on both sides, often symmetrically. Simple touch, position sense, and vibratory sensation are preserved in the same segments, because fibers that convey these sensations do not pass near the center of the spinal cord. More importantly, all sensory modalities above and below the involved segments remain intact. The most common condition affecting this region of the spinal cord is syringomyelia. The centrally located spinal cord cyst of syringomyelia is commonly found in the lower cervical and upper thoracic spinal cord. In this condition, the area of symmetric sensory loss often includes the arms and upper thorax, giving rise to the term *cape-like sensory loss*.

THE ANTERIOR PORTION OF THE SPINAL CORD CAN BE PREFERENTIALLY INVOLVED IN ISCHEMIC LESIONS

The anterior spinal artery perfuses the anterior two-thirds of the spinal cord, excluding the dorsal columns. Occlusion of this artery causes infarction

of the anterior spinal cord and results in loss of all sensory and motor functions below the level of the infarction, with the exception of vibratory and proprioceptive sensation, both of which are subserved by the intact dorsal columns. These fiber tracts receive their blood supply instead from the paired posterior spinal arteries.

PATHOLOGY BELOW THE L1 THROUGH L2 VERTEBRAL LEVEL AFFECTS THE CAUDA EQUINA BUT NOT THE SPINAL CORD

In normal adults, the spinal cord does not extend the entire length of the vertebral column. It does not extend below the second lumbar (L2) vertebral level. Because of this difference in length between the spinal cord and the vertebral column, spinal nerves in the cervical and upper thoracic regions enter or exit at almost right angles, but the lower thoracic, lumbar, and sacral spinal nerves originate at increasingly downward oblique angles. The lumbar and sacral spinal roots originate at the level of the lower thoracic and upper lumbar vertebrae and then descend in the spinal canal to the corresponding lower lumbar and sacral vertebrae, where they exit. This mass of descending spinal nerves within the spinal canal but below the spinal cord forms the cauda equina. Lesions within the spinal canal below the L2 vertebrae cannot involve the spinal cord; they involve only the cauda equina.

Lesions in this region of the spinal canal do not produce upper motor neuron symptoms or patterns of sensory loss related to interruption of ascending spinal cord fiber tracts. The lesions in this region produce symptoms that are related to involvement of lumbosacral spinal nerves, such as radicular pain, sensory loss involving the lower extremities, or significant bladder and sexual dysfunction. The last two abnormalities reflect involvement of the autonomic fibers contained in the sacral spinal nerve roots.

At the lower lumbar vertebral level, there is no concern about a spinal needle impaling the spinal cord. The spinal cord does not descend below the L2 level, allowing the removal of cerebrospinal fluid to be accomplished safely with a lumbar puncture of the lower lumbar spinal canal. In this procedure, a sterile needle is passed through the space between adjacent lower lumbar vertebrae into the spinal subarachnoid space (SAS). Although the component roots of the cauda equina are coursing through the SAS within the spinal canal at this vertebral level, an appropriately placed spinal needle nudges them aside without causing damage.

DIAGNOSTIC NEUROIMAGING PROCEDURES AND ELECTROPHYSIOLOGIC TESTS

Myelography had been the premier radiologic diagnostic modality used to investigate spinal cord disorders for several decades until the introduction of computed tomography (CT) and magnetic resonance imaging (MRI) scanning. Myelography involves the introduction of a radiopaque dye into the spinal fluid through a lumbar puncture so that it outlines the spinal cord, spinal nerves, and cauda equina. CT scanning is sometimes used, but MRI has emerged as the most definitive technique for imaging the spinal cord because of its excellent contrast and its ability to display the cord in axial and sagittal arrays.

Somatosensory evoked potentials (SEPs) are performed by stimulating a peripheral nerve in the leg or arm, typically the tibial or median nerve, and recording the evoked potential over the upper cervical region or scalp. The elapsed time between the application of the stimulus and the arrival of the evoked potential is recorded, as is the amplitude of the response. These potentials reflect passage of the evoked impulse through spinal cord pathways, largely the posterior columns. An abnormality within these pathways may affect the latency of the response or its amplitude. SEPs are useful in identifying subtle pathology of the spinal cord even in the absence of clinical symptoms. They are also used to monitor spinal cord function intraoperatively during procedures that involve significant manipulation of the spinal cord. Scoliosis surgery, during which there may be stretching of the spinal cord, is one example of a procedure during which SEPs are routinely performed.

TREATMENT OF SPINAL CORD INJURY

Current treatment strategies are aimed at preventing further injury and at subsequent rehabilitation. In cases of trauma, high-dose steroids are given to limit swelling. Compressive lesions may be treated surgically, and in cases of fracture or dislocation, the vertebral column may be stabilized surgically. Rehabilitative services such as locomotor training focus on optimizing remaining function, and other interventions aim at the prevention of complicating illnesses such as bladder infections and skin breakdown from immobility. Although not yet a clinical reality, considerable attention is being focused on the prospects of spinal cord regeneration and restoration.

of function. Strategies that employ the use of neurotrophic factors such as NGF, BDNF, and NT-3, agents that block factors such as Nogo A, and transplantation of various tissues including embryonic stem cells, Schwann cells, and olfactory ensheathing glial cells are currently being investigated.

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Charles R. Goodlett

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1. OVERVIEW

The cerebellum, aptly named from Latin meaning “little brain,” accounts for only about 10% of the total weight of the brain (about 150 g in the adult human male) but contains more than 50% of the brain’s neurons. The cerebellum is present in some form in all vertebrates. Its neurons and connectional anatomy are highly organized with a geometric regularity that repeats in a modular fashion over the entire cerebellum. Three major functional subdivisions can be identified in the mammalian cerebellum, corresponding with different phylogenetic origins: the vestibulocerebellum (archicerebellum), spinocerebellum (paleocerebellum), and cerebrocerebellum (neocerebellum). The cellular architecture of these divisions is remarkably uniform, but they differ in the source of their afferent input (vestibular, spinal, and corticopontine projections, respectively) and the targets of their efferent regulation. Because there are no associational/commissural connections between different regions of the cerebellum, different portions of the cerebellum that have segregated afferent and efferent connections represent separate modules of functional circuits that operate in parallel.

The cerebellum receives considerable sensory input from skin, joints, muscles, vestibular system, and visual system, yet functions most notably to regulate motor function. In regulating movement and action, the cerebellum contributes comparatively modest direct connections to brain-stem motor nuclei that give rise to descending spinal pathways (red nucleus, vestibular nuclei). In contrast, the cerebellum projects heavily back to all major motor-control regions, and to prefrontal and limbic cortical regions, via thalamic nuclei.

The cerebellum contributes to five broad domains of function. It maintains balance and equilibrium during standing and locomotion and adjusts gaze when the head is moving to keep the retinal image stable. It monitors and corrects ongoing or future movements by comparing intended movement with actual performance. It coordinates the timing and execution of complex voluntary movements. It mediates motor learning and motor skill acquisition through its capacity for experience-dependent modification of synaptic transmission. Recent evidence indicates that it also makes important contributions to nonmotor, cognitive functions such as verbal working memory, planning, and executive control.

The organization of the circuitry interconnecting the cortical motor territories with the cerebellum provides the structural basis for its comparator function. Topographically segregated circuits

(“cortico-ponto-cerebellar” loops) carry afferent input from cortical regions involved in motor programming to specific regions of the cerebellum (via the pons), which link back to the motor territories by efferent projections via the thalamus. These loops help compute disparities between intended movements (signaled through the “feedforward limb” from the cortex via cortico-ponto-cerebellar projections) with ongoing data about the status of motor performance (from sensory input) and generate error signals back to the cortex that permit correction of ongoing or subsequent motor programs (signaled through the “feedback limb” from the cerebellum to the cortex via the thalamus). Notably, the cerebellum performs these complex functions rapidly and automatically, without requiring conscious effort on the part of the individual.

Similar loops between prefrontal associational cortex and cerebellum have been demonstrated, likely mediating cerebellar contributions to some nonmotor functions (Kelly and Strick, 2003). Functional neuroimaging and neuropsychological studies in humans provide support for a role of the cerebellum in certain cognitive processes (Schmahmann, 1997; Desmond and Fiez, 1998). The cerebellum is essential for various forms of motor learning, which in turn depend on synaptic modification in cerebellar circuitry through experience-dependent synaptic plasticity. Cerebellar damage typically does not produce sensory impairment or decreased muscle strength. Rather, depending on the region or fiber tracts involved, cerebellar damage can interfere with balance, equilibrium, or gait, with the timing, accuracy, or coordination of movements, with the acquisition of motor learning and motor skills, or with certain aspects of cognitive function.

2. THE GENERAL ORGANIZATION OF THE CEREBELLUM

2.1. Gross Morphology of the Cerebellum

The cerebellum occupies the posterior cranial fossa, which is separated from the occipital lobes of the cerebral hemispheres by a conspicuous transverse extension of the dura known as the *tentorium cerebelli*. The cerebellum is the largest part of the hindbrain. It overlies a substantial portion of the posterior surface of the pons and medulla oblongata. Developmentally, the cerebellum is derived first from the germinal cells of the ventricular epithelium of the fourth ventricle (part of the alar lamina) and, later in development, from the rhombic lip, germinal regions associated with sensory precursors.

The cerebellum is a highly convoluted, ovoid-shaped structure that consists of two *cerebellar hemispheres* joined by a narrow, median longitudinal strip in the middle, the *vermis* (Fig. 1). On the superior surface of the cerebellum, the vermis is seen as an elevated region at the midline, with the cerebellar hemispheres gently sloping in a lateral fashion. In contrast, the inferior surfaces of the hemispheres are convex, and the midline vermis is recessed and forms the floor of a deep crevice, the *vallecula*. *In situ*, the vallecula is occupied by a vertical extension of dura mater known as the *falx cerebelli*, which houses a venous channel for drainage of the cerebellum. Near the midline inferiorly, the cerebellum surrounds the dorsolateral aspect of the medulla with two swellings, the *cerebellar tonsils*. Occasionally, the cerebellar tonsils are useful in diagnosing elevated intracranial pressure, as they tend to herniate through the foramen magnum as a result of this condition. The region of the hemispheres immediately adjacent to the midline vermis is known

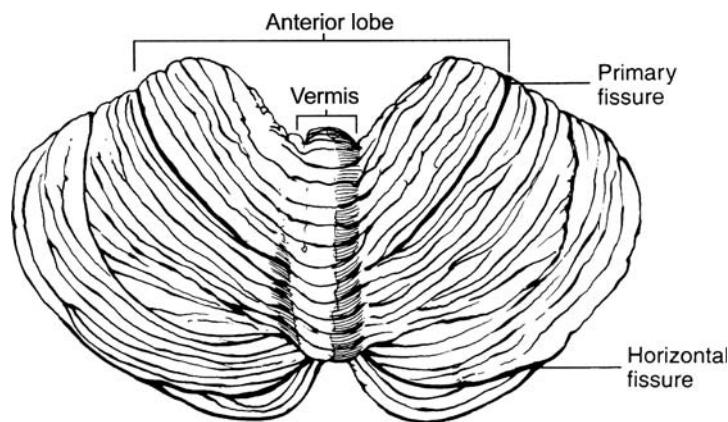


Fig. 1. The superior surface of the cerebellum.

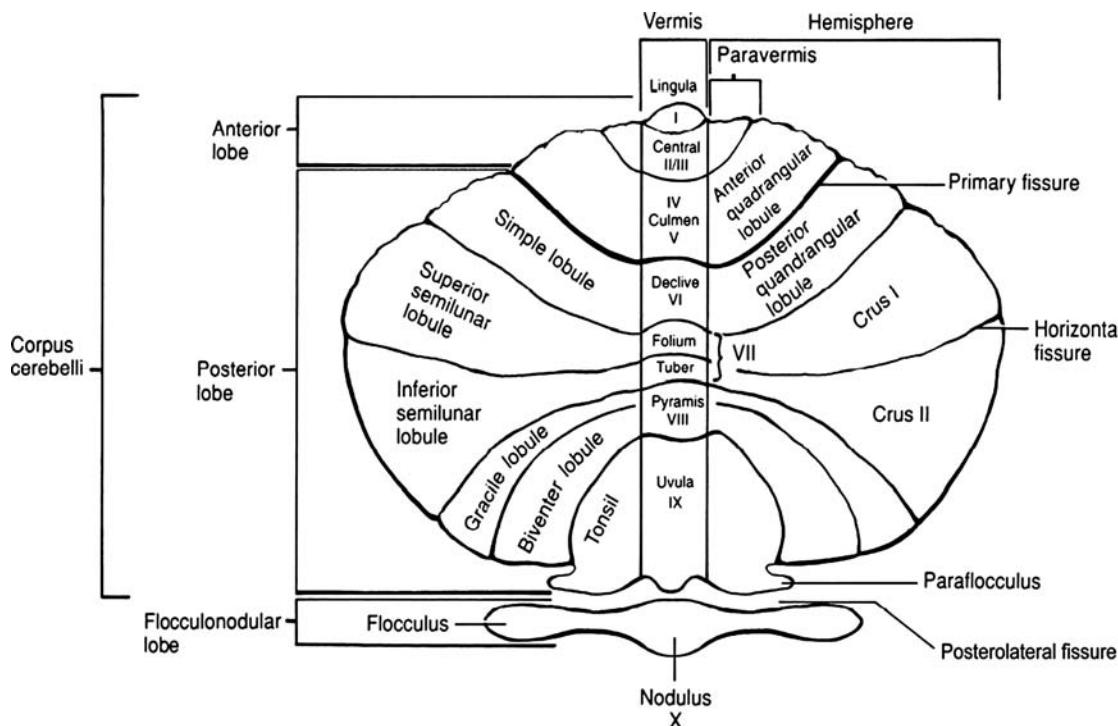


Fig. 2. Schematic of the flattened cerebellum showing the fissures, lobes, and lobules of the cerebellum. The terms on the left side of the diagram refer to terminology used for the human cerebellum. Terms on the right side refer to terminology used for animals. The Roman numerals designate the 10 lobules of the vermis. (The drawing is modified from Larsell, 1951.)

as the *intermediate zone* or *paravermis*. The primary mass of the cerebellum, excluding the flocculonodular lobe, is called the *corpus cerebelli* (Fig. 2).

The surface of the cerebellum contains a distinct, three-layered cortex composed of gray matter. Deep to the cerebellar cortex is an extensive central core of white matter consisting of afferent and efferent axons of the cerebellum. In the midline region, this deep white matter forms the roof of the fourth ventricle. Embedded within the deep white matter near the midline are four pairs of *deep cerebellar nuclei*, which provide the vast majority of the output axons that leave the cerebellum (see Fig. 6). More laterally and ventrally, the deep white matter separates into three bilateral pairs of white matter tracts, the cerebellar peduncles (superior, middle, and inferior), which concomitantly provide both a physical attachment of the cerebellum to the brain stem and a means of communication between the cerebellum and the rest of the central nervous system (see Fig. 5). A thin, white lamina, the *superior medullary velum*, stretches between the superior cerebellar peduncles, forming the cranial portion of the roof of the fourth ventricle (Fig. 3). There is also a small *inferior medullary velum*, which stretches between the inferior portion of the cerebellum near the midline and the dorsal aspect of

the medulla. This thin membrane forms the caudal extent of the roof of the fourth ventricle and contains a midline opening known as the *foramen of Magendie*. This opening provides an outlet for the passage of cerebrospinal fluid from the fourth ventricle into the subarachnoid space.

2.2. The Lobes, Lobules, and Fissures of the Cerebellum

The surface of the cerebellum is conspicuously different from the cerebrum, most notably by the appearance of many thin, transversely oriented, leaf-like structures called *folia* and their accompanying parallel fissures (Fig. 1). This appearance of the surface of the cerebellum is caused by extensive folding of the cerebellar cortex, resulting in approximately 85% of its surface being hidden. Thus, despite the comparatively small size of the cerebellum, the linear extent of the surface area of the cerebellar cortex (and its underlying gray matter) is surprisingly large. For example, if the cerebellar cortex were laid out into a flat sheet, it would extend over a meter in length and have an area of approximately 50,000 mm².

Although the cerebellum is divided by many transversely oriented fissures, only the *primary fissure* and

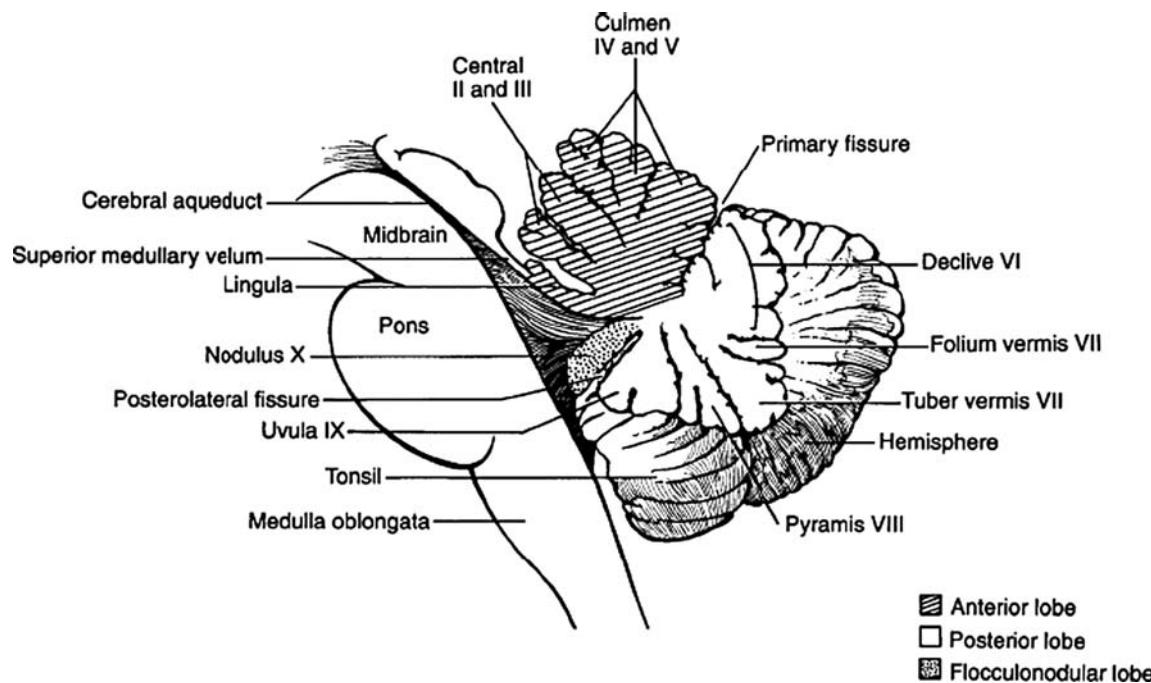


Fig. 3. A midsagittal section through the cerebellar vermis and brain stem.

the *posterolateral fissure* are significant. They divide the cerebellum into three main lobes: the *anterior lobe*, the *posterior lobe*, and the *flocculonodular lobe* (Fig. 1, Fig. 2, Fig. 3, Fig. 4, and Fig. 5). The anterior lobe is the superior part of the cerebellum, rostral to the V-shaped *primary fissure* (Fig. 1, Fig. 2, and Fig. 3). The posterior lobe (sometimes called the middle lobe) is the largest of the three lobes and is positioned dorsally between the primary fissure and posterolateral (pre-nodular) fissure (Fig. 3). Immediately rostral to the posterolateral fissure is the smallest lobe, the flocculonodular lobe (Fig. 2, Fig. 3, Fig. 4, and Fig. 5). The flocculonodular lobe is composed of the midline nodulus and the two laterally placed flocculi. The small, semidetached portions of each cerebellar hemisphere that extend to merge with the flocculi are the nodulus, forming the rostral pole of the inferior vermis. The deep horizontal fissure (Fig. 1 and Fig. 2) is easily identified, but it has no apparent functional significance.

The lobes are divided into lobules, which in turn are further subdivided into many folia. The lobes, lobules, and most of the folia run continuously in a predominately transverse but somewhat curved direction, from hemisphere through the vermis and to the other hemisphere. Traditionally, the lobules are identified by names. In the vermis, the lobules are also identified with Roman numerals (I to X) (Larsell, 1951). The lobular organization can best be distinguished by viewing a midsagittal section through the

vermis (Fig. 3), as this is the only region in which all 10 lobules are present. For the most part, the separate lobules have no known functional significance; thus, they are of limited use clinically. However, they are often used for descriptive purposes in experimental studies.

2.3. The Cerebellum Has Three Major Functional Divisions

The cytoarchitecture and structural organization of the neurons of the cerebellar cortex are the same over the entire cerebellum. Consequently, differences in function of different parts of the cerebellum arise from differences in the fiber connections, most prominently from differences in the sources of afferent fibers. Based on a combination of criteria, including its phylogenetic development and from experimental studies of fiber connections, the cerebellum can be divided into three basic functional divisions (Fig. 4).

The flocculonodular lobe comprises the *archicerebellum*, or *vestibulocerebellum*, and is phylogenetically the oldest division and varies little across mammalian species. As its name implies, it is related functionally to the vestibular system, receiving direct connections from the vestibular nerve, and has reciprocal connections with brain-stem vestibular nuclei. Input from the visual system also projects to the vestibulocerebellum. It controls posture and balance while standing and walking (Morton and Bastian, 2007) and coordinates

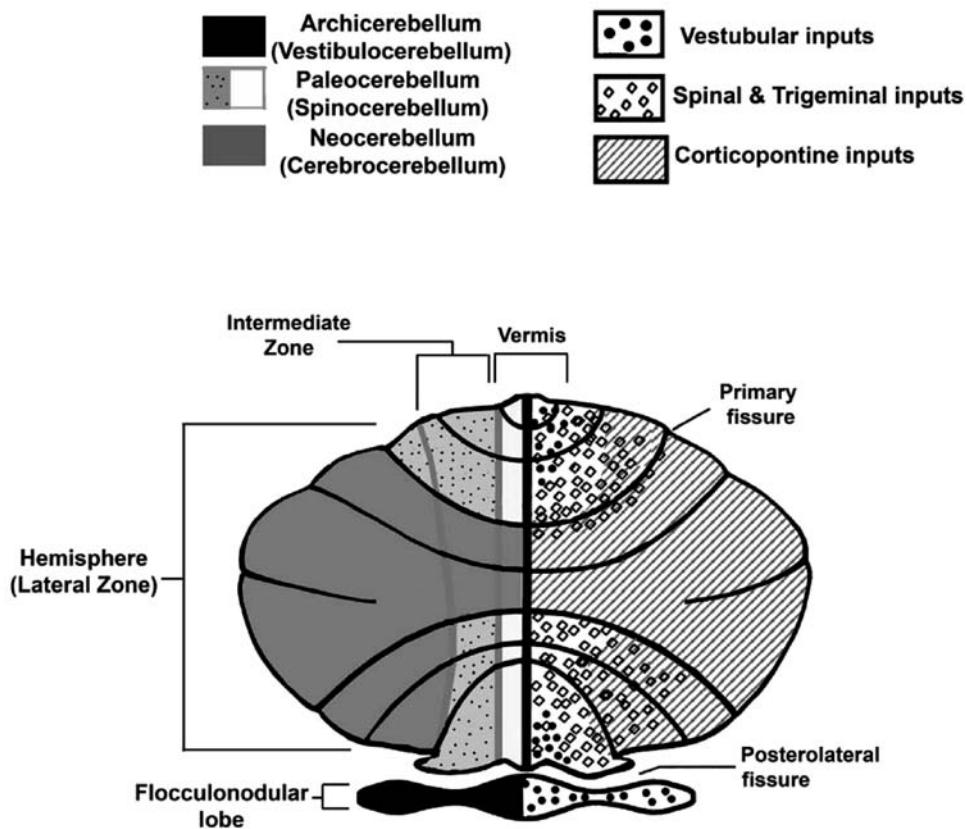


Fig. 4. The functional divisions of the cerebellum are based on phylogenetic development (shown in left hemisphere) and the termination of afferents to the cerebellar cortex (shown in right hemisphere). (Based on Dow, 1942, and Brodal, 1981.)

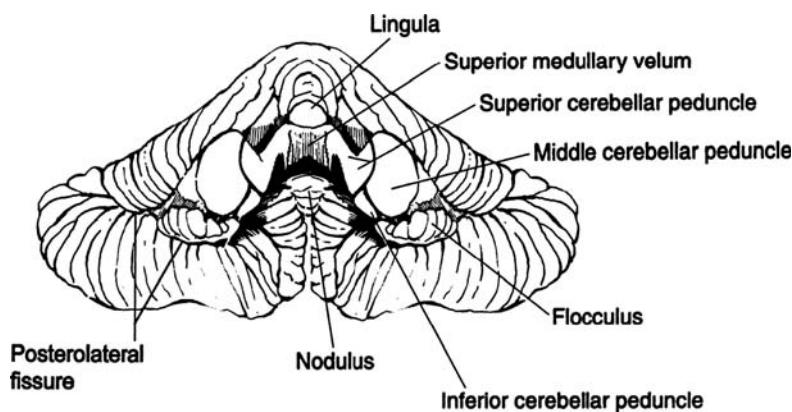


Fig. 5. The anteroinferior surface of the cerebellum, illustrating the cut ends of the three cerebellar peduncles.

eye movements with movements of the head through its connections to portions of the vestibular nuclei that control the extraocular muscles (Ito, 1984).

The *paleocerebellum*, or *spinocerebellum*, is phylogenetically the next region of the cerebellum to appear. It consists of most of the vermis (anterior and posterior, excluding the portion just posterior to primary fissure), the intermediate zone (also called the paravermis), and

anterior lobe (Fig. 4). The vermis receives prominent input from the spinal cord (trunk/limbs) and from the trigeminal system (head/neck). The intermediate zone can be identified only on the basis of its efferent connectivity (to the interposed nuclei, described below), not macroscopically. The intermediate zone is a main region of converging input from the spinal systems and the motor cortex, likely providing the requisite circuitry

for the comparing discrepancies between intended movement (from cortical input) and actual movement (from peripheral sensory input). The spinocerebellum receives multiple inputs from the spinal cord, mainly from two spinal sources. Spinal neurons that are monosynaptically activated by fast-conducting sensory afferents (muscle spindles, tendon organs, low-threshold mechanoreceptors) convey information from proprioceptors and skin to the spinocerebellum. Spinal interneurons that receive input from descending motor pathways also project to the spinocerebellum, likely conveying information about motor programs that reach the spinal level. The spinocerebellum uses this extensive, topographically organized input from the limbs and head/neck to control posture, muscle tone, and synergy during stereotyped movements such as walking or reaching and grasping.

The *neocerebellum* (*cerebrocerebellum*, *pontocerebellum*), consists primarily of the large, lateral parts of the hemispheres' posterior lobes. It is the largest and phylogenetically the youngest part of the cerebellum, and is expanded in humans and higher primates. The neocerebellum receives projections from the neurons in the contralateral pontine nuclei and inferior olfactory nuclei, which in turn receive direct projections from cerebral cortex involved in motor programming. Efferent projections of the neocerebellum, projecting back to the contralateral motor regions of the cortex, are by way of the deep nuclei and the thalamus. These circuits form the "cortico-ponto-cerebellar loops" that provide the structural basis for regulation of cortical motor programs. The neocerebellum regulates the timing and accuracy of planned movements and coordinates the execution of skilled movements, for example, of the fingers (Glickstein et al., 2005). The neocerebellum has also been implicated in regulation of speech and language (Ackermann et al., 2007; De Smet et al., 2007), acquisition of motor skills, and learning and execution of accurate, nonstereotyped actions (Thach, 1998). Regulation of nonmotor (associational) cortical regions is also now established, including cortico-ponto-cerebellar loops between the prefrontal cortex and the cerebellum (Schmahmann and Pandya, 1997; Kelly and Strick, 2003). Based in part on these functional circuits, the cerebellum has been implicated in regulation of various cognitive and affective processes (Schmahmann, 1997), including verbal working memory (Chen and Desmond, 2005; Desmond et al., 2005; Ravizza et al., 2006), planning and executive functioning (Bellebaum and Daum, 2007), and emotional processes (Schmahmann, 2004).

2.4. The Cerebellum Is Organized into Longitudinal Zones

The structural and functional organization of the cerebellum is now recognized to be far more precise than the large medial-to-lateral divisions of vermis, intermediate zone, and lateral hemispheres described above. Seven morphologically distinct longitudinal (translobular) zones that run parallel in the parasagittal plane can be identified in the cerebellar cortex, in some cases traversing continuously the entire rostro-caudal extent of the cerebellum. Each zone receives climbing fibers from a specific area of the inferior olive, and each projects Purkinje cell axons to specific, targeted portions of the deep cerebellar nuclei (or vestibular nuclei). This grouping into longitudinal compartments defined by specific, spatially segregated modules of olfactory inputs, Purkinje cells, and efferent projections to the specific areas of the deep nuclei establishes a modular organization of cerebellar function (Voogd and Glickstein, 1998; Ito, 2006). Similarly, parasagittal stripes have been identified using histochemistry for specific markers such as acetylcholinesterase or aldolase C (Zebrin II). These longitudinal stripes loosely correspond with the anatomically based zones, but the overlap is not very strict.

2.5. The Cerebellar Peduncles Convey Fibers In and Out of the Cerebellum

The cerebellum is connected to the posterior surface of the lower three segments of the brain stem by three thick pairs of fiber bundles: the *superior*, *middle*, and *inferior cerebellar peduncles* (Fig. 5). All of the inputs and outputs of the cerebellum are routed through these peduncles (Table 1).

The *superior cerebellar peduncle* (*brachium conjunctivum*) physically connects the cerebellum with the lower portion of the midbrain. It contains the principal efferent pathways leaving the cerebellum from the globose, emboliform, and dentate nuclei. The superior cerebellar peduncle also conveys afferent fibers into the cerebellum from the ventral spinocerebellar tract, the tectocerebellar tract, the rubrocerebellar tract, and a small noradrenergic projection from the locus coeruleus.

The *middle cerebellar peduncle*, or *brachium pontis*, is the largest of the peduncles and connects the pons with the cerebellum. It exclusively carries afferent fibers provided by the (cortico) pontocerebellar tract that projects from the *contralateral* pontine nuclei to the cerebellum. Because the pontine nuclei receive direct projections from many regions of the ipsilateral cerebral cortex, the middle cerebellar peduncle relays

Table 1
Principal Inputs to the Cerebellum

Tracts	Origin	Termination zone	Peduncle
Ventral spinocerebellar	Spinal cord	V,P	Superior
Tectocerebellar	Sup. and inf. colliculi	V,P	Superior
Aminergic	LC, RP, VMT	V,P,L	Superior
Corticopontocerebellar	Pontine nuclei	V,P,L	Middle
Dorsal spinocerebellar	Spinal cord	V,P	Inferior
Olivocerebellar	Inf. and access. olive	V,P,L	Inferior
Cuneocerebellar	Lat. cuneate nucleus	V,P	Inferior
Vestibulocerebellar	Vestibular organ, Vestibular nuclei	F,N,U F,V	Inferior
Reticulocerebellar	Reticular formation (RF)	V,P	Inferior
Arcuatocerebellar	Arcuate nucleus	F	Inferior
Trigeminocerebellar	Trigeminal nerve (CN V)	V,P	Inferior

Abbreviations: V, vermal zone; P, paravermal zone; L, lateral zone; F, flocculus; N, nodulus; U, uvula; LC, locus coeruleus; RP, raphe nuclei; VMT, ventral mesencephalic tegmentum; Inf., inferior; Sup., superior; Lat., lateral; access., accessory; CN V, cranial nerve V.

information to the cerebellum that originates in the contralateral cerebral cortex.

The *inferior cerebellar peduncle* is a large bundle of input and output fibers that connects the cerebellum with the medulla oblongata. Seven distinct afferent pathways have been identified that enter the cerebellum through the inferior cerebellar peduncle (Table 1). It is composed of two parts, the larger *restiform body*, which conveys afferent fibers to the cerebellum from spinal cord and brain stem, and the smaller, medially positioned *juxtarestiform body*, which carries a small bundle of vestibular fibers to and from the cerebellum. Important efferent fibers that pass out through the inferior cerebellar peduncle are the cerebellovestibular, cerebello-olivary, and the cerebelloreticular fibers.

2.6. The Deep Cerebellar Nuclei Provide Most of the Output of the Cerebellum

In humans and the highest primates, there are four pairs of nuclei embedded within the white matter of the cerebellum. From medial to lateral, they are the *fastigial*, *globose*, *emboliform*, and *dentate nuclei* (Fig. 6). Often, the emboliform and globose nuclei are combined into one cell mass on each side and called the *interposed* or *interpositus nucleus*. Inputs to the deep cerebellar nuclei are derived from two sources, the Purkinje cells of the cerebellar cortex and extracerebellar sources (including collaterals from climbing fibers and some mossy fibers that innervate the cerebellar cortex). Purkinje cells provide the sole output from the cerebellar cortex. Purkinje cells of the flocculonodular lobe and a few

from the vermis project directly to the vestibular nuclei. However, the vast majority of Purkinje cell axons do not leave the cerebellum but synapse in the deep cerebellar nuclei.

The deep cerebellar nuclei, together with the vestibular nuclei, relay the entire output from the cerebellum to other parts of the central nervous system. The neurons of the deep cerebellar nuclei are tonically active and provide excitatory drive to their targets. The *fastigial nucleus* is sometimes called the “rooftop nucleus” because it is located near the midline in the roof of the fourth ventricle, and in lower mammals it is sometimes termed the medial nucleus. The fastigial nucleus receives input from Purkinje cells in the vermis and gives rise to three efferent bundles that project *bilaterally* through the juxtarestiform body of the inferior cerebellar peduncle primarily to the vestibular nuclei and reticular formation (RF). The *globose nucleus* actually consists of two or

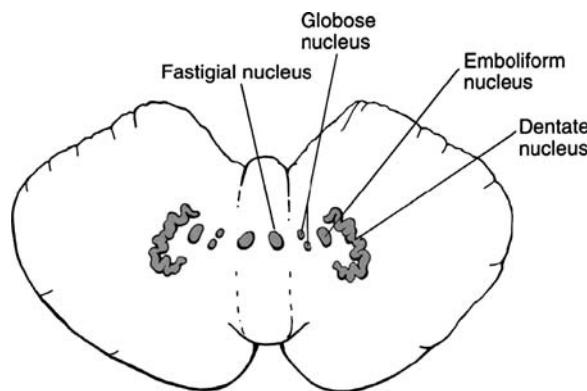


Fig. 6. A diagram demonstrating the four pairs of deep cerebellar nuclei. (Based on Gluhbegovic and Williams, 1980.)

three very small clumps of cells. The *emboliform nucleus* is a small oval nucleus that is lateral to the globose nucleus and medial to the concave hilus of the dentate nucleus. The globose and emboliform nuclei receive inputs from Purkinje cells in the intermediate zone. The efferent fibers of both the globose and the emboliform nuclei exit the cerebellum through the superior cerebellar peduncle and project to numerous motor control areas in the brain stem, but primarily to the red nucleus on the *contralateral* side.

The *dentate nucleus* is the largest, most laterally placed, and most conspicuous of the deep cerebellar nuclei. In cross section, it has the appearance of a crumpled band of cells, similar to that of the inferior olive. In keeping with its connectional association with the lateral cerebellar hemispheres (that are greatly enlarged in higher primates), the dentate nucleus becomes dramatically convoluted in man compared with that of non-primates. Careful dissection has shown that the dentate nucleus is actually composed of numerous nodules or fingers of gray matter (Glubbegovic and Williams, 1980). Each dentate nucleus receives topographically organized input from Purkinje cell axons from the lateral portions of the ipsilateral cerebellar hemispheres. The efferent fibers from the dentate nucleus represent the primary component of the superior cerebellar peduncle. The main output projects from the hilus of the dentate nucleus to the *contralateral* ventral lateral (VL) nucleus of the thalamus (with a smaller projection

to the ventral anterior (VA) and dorsomedial (DM) nuclei of the thalamus). In addition, there are also small projections to the red nucleus, RF, and oculomotor nucleus, all on the *contralateral* side (see Fig. 13).

In addition to distributing cerebellar information to other parts of the brain, collateral axons from all deep cerebellar nuclei project back to the same areas of the cerebellar cortex from which they received Purkinje cell projections. The deep nuclei also receive collateral inputs from climbing fibers and mossy fibers that project to the cerebellar cortex. Thus, the outputs from the deep cerebellar nuclei represent the output of the feedback loops from the cerebellum to other areas of the central nervous system that are associated with the control of cerebellar function, including the cerebellum itself. A generalized summary of afferent, intracerebellar, and efferent projections of the cerebellum is provided in Fig. 7.

2.7. The Blood Supply to the Cerebellum Is Derived from Three Arteries

The cerebellum receives its blood supply from three paired arteries (Fig. 8). Two of these arteries supply the inferior surface of the cerebellum. The *posterior inferior cerebellar arteries* (PICA) arise from their respective vertebral arteries just before the latter join to form the basilar artery. The PICAs supply the majority of the inferior surface of the cerebellum and include branches that supply the inferior vermis, dorsolateral medulla, and restiform body. The

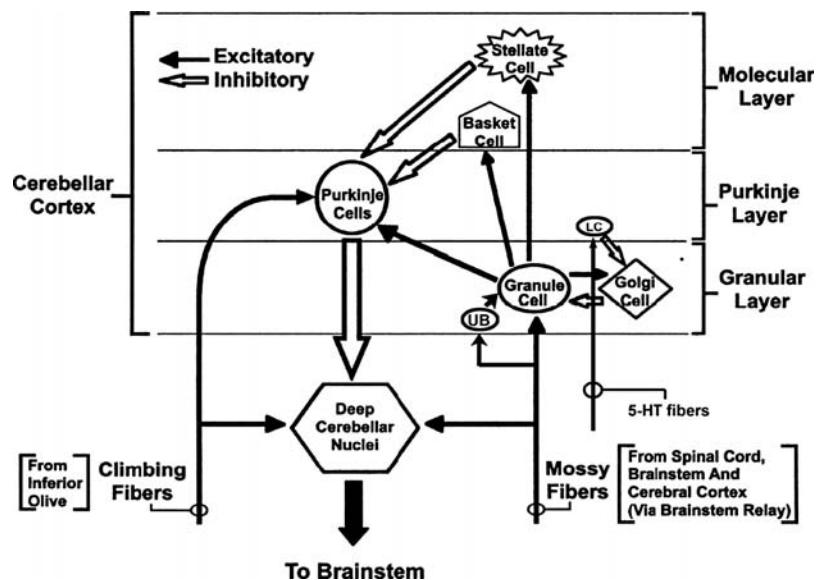


Fig. 7. Simplified diagrammatic representation of the afferent, intracerebellar, and efferent circuitry of the cerebellum, illustrating excitatory and inhibitory components. LC, Lugaro cell; UB, unipolar brush cell; 5-HT, serotonergic fibers; all other cell types and fibers are labeled in full.

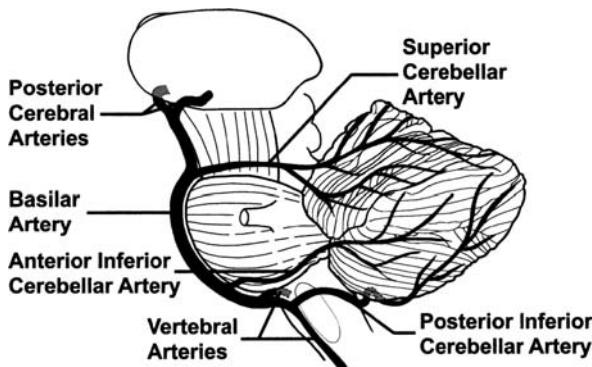


Fig. 8. Lateral view of the cerebellum and brain stem, illustrating the three paired arteries that supply the cerebellum.

anterior inferior cerebellar arteries (AICA) typically arise bilaterally near the caudal end of the basilar artery and supply the more anterior portions of the inferior cerebellum (e.g., the flocculus, the caudal portion of the dentate nucleus, and the middle cerebellar peduncle). The *superior cerebellar arteries* arise bilaterally near the rostral end of the basilar artery. They supply the superior (dorsal) portion of the cerebellum, most of the deep cerebellar nuclei, the rostral portion of the middle cerebellar peduncle, and the superior cerebellar peduncle. The venous drainage of the cerebellum is conducted via various venous sinuses and the great cerebral vein.

3. THE CYTOARCHITECTURE OF THE CEREBELLAR CORTEX IS CONSPICUOUSLY UNIFORM

3.1. Three Well-Defined Layers of the Cerebellar Cortex Emerge During Development

The cerebellar cortex is a highly convoluted sheet of gray matter about 1 mm thick. Unlike the cerebral cortex, the cerebellar cortex exhibits uniformity both in thickness and in organization. It exhibits a relatively simple, but highly organized, geometric cytoarchitecture that is remarkably similar in all mammalian species. The cerebellum has three distinct layers, and it contains seven types of neurons. The neurons exhibit a remarkable degree of uniformity in their organization throughout the cortex, both with respect to the transverse and longitudinal axes and to the pial surface (Fig. 9).

The outermost layer just beneath the pial surface is known as the *molecular layer*. It is packed with dendrites and axons, but relatively few neuronal cell bodies (only *stellate cells* and *basket cells*). The middle layer, the *Purkinje cell layer*, is composed of a single layer of *Purkinje cell* bodies in the mature

cerebellum. The deepest of the three cerebellar cortical layers is the *granular layer*. It is the thickest layer, formed mainly by the enormous number of small, densely packed *granule cells*. The granular layer also contains *Golgi cells*, located just deep to the Purkinje cell layer, and *unipolar brush cells* and *Lugano cells*.

The cerebellum develops from two germinal zones (Sotelo, 2004). One is the ventricular epithelium of the cerebellar anlage (or primordium), which generates all cerebellar neurons except the granule cells. The second is the rostral rhombic lip of the hindbrain zone, which provides the proliferating precursors that form the *external germinal layer* that generates granule cells; the rhombic lip is also the source of neurons forming the precerebellar nuclei (inferior olive, pontine nuclei).

During embryonic development, a relatively small pool of progenitor cells in the ventricular germinal zone of the cerebellar anlage first gives rise to the neurons of the deep cerebellar nuclei, followed closely by the generation of Purkinje cells. The postmitotic Purkinje neurons migrate toward the pial surface dorsal to the roof of the fourth ventricle, past the neurons forming the deep cerebellar nuclei, where they form clusters that eventually fuse. Subsequently, other precursor cells—which are still capable of proliferating—migrate from the ventricular zone toward the emerging cerebellar cortex and eventually form unipolar brush cells, Golgi cells, basket cells, stellate cells, and glia. The Purkinje neurons eventually settle into a single layer, and the subsequent arrival of the climbing fibers and formation of synaptic contacts on short-lived perisomatic processes of the Purkinje cells coincides with the stimulation of dendritic outgrowth into the molecular layer and their innervation by developing parallel fibers of the late-developing granule cells.

After formation of the primordial cerebellum, the transient *external germinal layer* (EGL) [or *external granular layer* as originally named by Ramon y Cajal] is formed that is several cells thick between the pial surface and the molecular layer. The EGL is formed by migration of proliferating progenitor cells from the rostral rhombic lip, which move dorsorostrally to cover the full extent of the surface of the emerging cerebellar primordium (Altman and Bayer, 1997; Hatten, 2002; Sotelo, 2004). These neuroblasts establish the EGL proliferative zones that, later in development, give rise to granule cells (Alder et al., 1996). It was originally believed that the EGL also gave rise to stellate, basket, and Golgi cells, but more recent cell lineage studies using viral tags indicate that these three interneuronal populations likely arise

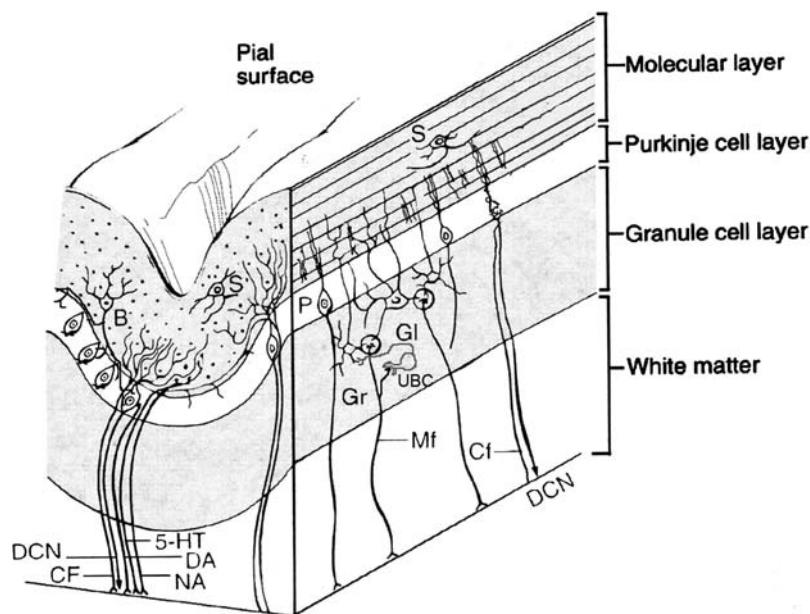


Fig. 9. Schematic of the basic cytoarchitecture of the cerebellar cortex represented by single cerebellar folium. Three types of inputs—climbing fibers (Cf), mossy fibers (Mf), and aminergic fibers (DA, dopaminergic; 5-HT, serotonergic; NA, noradrenergic)—are illustrated. Five main types of neurons are identified. P, Purkinje cell; Gr, granule cell; B, basket cell; S, stellate cell; UBC, unipolar brush cell. In addition, the glomerulus (Gl) and output to deep cerebellar nuclei (DCN) are illustrated. Lugano cells (and their innervation by 5-HT fibers) are not depicted. The orientation of the diagram is such that the surface on the left side represents the transverse plane, and the right side of the diagram represents the longitudinal plane of a folia. (Based in large part on descriptions and drawings from Eccles et al., 1967; Ito, 1984; Ito, 2006.)

from a common pool of stem cells of the ventricular epithelium near the fourth ventricle (Zhang and Goldman, 1996; Sotelo, 2004). The cellular proliferation in the EGL is governed, in part, by molecular signaling from the Purkinje cells (Sotelo, 2004). During granule cell generation, after a terminal division the undifferentiated postmitotic granule cells move from the superficial (proliferative) zone of the EGL to the deeper zone of the EGL where they begin to differentiate. The differentiating granule cells migrate down through the molecular and Purkinje-cell layers to reach the granular layer, leaving trailing axonal processes that bifurcate to form the parallel fibers that extend along the longitudinal axis of the folia, with the later-formed axons stacking on top of (superficial to) the earlier formed axons. Granule cell migration is guided by interactions with the scaffold-like processes of Bergman glia (Hatten, 2002; Sotelo, 2004; Yue et al., 2005). The granule cells born earliest are found in the deeper portions of the granular layer, with later-born granule cells settling in more superficial portions (Altman and Bayer, 1997). As granule cell neurogenesis progresses toward completion, the EGL diminishes and then disappears.

The generation, migration, differentiation, and synaptogenesis of the neurons of the cerebellar cortex

occur over a protracted period that, in humans, extends through the third trimester and over the first 2 years of life (Dobbing, 1981). This relatively late period of rapid cerebellar growth, involving acquisition of granule cells, elaboration of Purkinje cell dendrites, and formation of the synaptic circuitry and synaptogenesis in the cerebellar cortex (Zecevic and Rakic, 1976), likely makes experience-dependent modifications in cerebellar circuitry in early infancy an important part of its functional maturation.

3.2. Neuronal Types in the Cerebellar Cortex

3.2.1. Purkinje Cells

Purkinje cells are highly differentiated neurons whose cell bodies form a monolayer sandwiched between the molecular and granular layers. The large, flask-shaped cell bodies give rise to large, fan-shaped dendritic arbors that fill the molecular layer in a distinctive pattern that is broad in the transverse plane of the folia and distinctly flattened in the horizontal plane (Fig. 7 and Fig. 9). Purkinje cells are often called the principal neurons of the cerebellum because they provide the sole output from the cerebellar cortex; all other neurons in the cerebellar cortex are intrinsic neurons. As a Purkinje cell axon passes through the granule cell layer, it gives

off one or more collaterals directed back near the Purkinje cell layer. Purkinje cell axons are myelinated, and the large majority synapse on neurons in the deep cerebellar nuclei, on which they provide the large majority of the total number of synapses, all of which are inhibitory. Gamma-aminobutyric acid (GABA) is the neurotransmitter released from Purkinje cell terminals. The tonically active excitatory neurons of the deep nuclei provide the large majority of projections out of the cerebellum, so the inhibitory control exerted by the Purkinje cells on these output neurons can powerfully regulate and sculpt their efferent activity to other brain sites. Purkinje cell axons from the flocculonodular lobe, and a few from the vermis, bypass the deep cerebellar nuclei and synapse directly onto neurons in the vestibular nuclei.

3.2.2. Granule Cells

There is an immense population of cerebellar granule cells. Cerebellar granule cells are the smallest, most densely packed, and by far the most numerous neuron type in the entire brain. They form the mass of the thick granular layer deep to the Purkinje cell layer and give rise to the heavy *parallel fiber* projections in the molecular layer that run medial-to-lateral along the long axis of the folia. The parallel fibers of the granule cells provide the more numerous of the two principal inputs to the Purkinje cells, and granule cells are the only intrinsic neurons of the cerebellum to contribute excitatory input to Purkinje cells. The claw-like terminal branches of granule cell dendrites act as a functional relay to enable mossy fiber information to reach the cerebellar cortex. The small-diameter, nonmyelinated granule cell axons pass superficially through the Purkinje cell layer into the molecular layer, where the axons bifurcate into the characteristic T-shaped junction. The two branches travel 1 to 1.5 mm in opposite directions horizontally through the molecular layer in the direction of the long axis of the folium, side by side with many thousands of other similar fibers; hence their name, parallel fibers (Fig. 7, Fig. 9, and Fig. 10). There is a geometric relationship between the position of the granule cell body and the position of the parallel fiber in the molecular layer resulting from the developmental patterns of granule cell generation, migration, and axon outgrowth. The more superficial the granule cell is in the granular layer (i.e., the later it is born), the more superficial are its parallel fibers in the molecular layer. A single parallel fiber makes synaptic contacts with the dendrites of numerous Purkinje cells that are aligned in a row along the long axis of the folium (a parallel-fiber “beam”). This

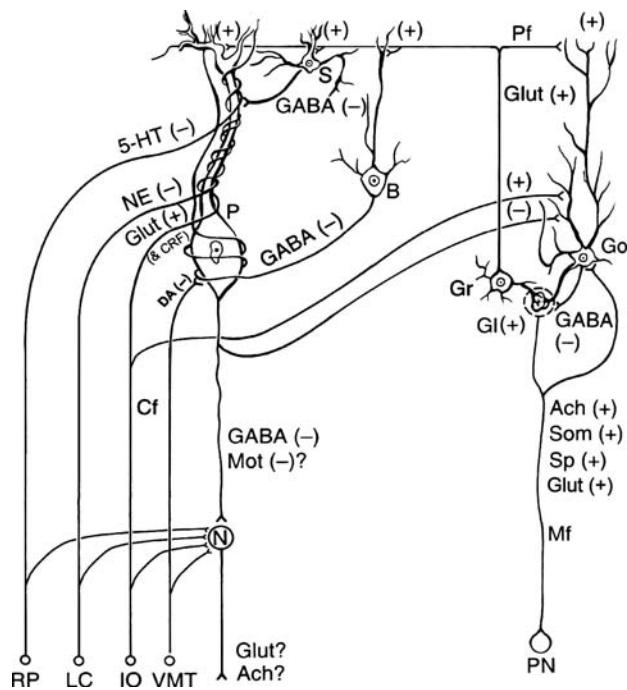


Fig. 10. The essential circuitry of the cerebellar cortex, depicting predominant neurotransmitters involved. P, Purkinje cell; Go, Golgi cell; Gr, granule cell; B, basket cell; S, stellate cell; Cf, climbing fiber; Mf, mossy fiber; Pf, parallel fiber; GI, glomerulus; N, deep cerebellar nuclei; IO, inferior olive; LC, locus coeruleus; PN, precerebellar neurons (from various sources); RP, raphe nuclei; VMT, ventral mesencephalic tegmentum; Ach, acetylcholine; CRF, corticotropin releasing factor; DA, dopamine; GABA, gamma-aminobutyric acid; Glut, glutamate; Mot, motilin; NE, norepinephrine; SOM, somatostatin; SP, substance P; 5-HT, serotonin. (Based in large part on Ito, 1984; 2006.)

organization allows each granule cell to synapse with the dendritic spines of several hundred Purkinje cells. Parallel fibers also make synaptic contact with basket, stellate, and Golgi cells. The functional output of granule cells is excitatory, and their neurotransmitter is believed to be glutamate.

3.2.3. Basket Cells

Basket cells are small neurons found in the deeper parts of the molecular layer near the Purkinje cell bodies. Their dendritic trees are unremarkable except that they are oriented as thin wafers; they are broad only in the transverse plane, similar to the orientation of the dendritic trees of Purkinje cells. Basket cells derive their name from the characteristic pericellular basket that their axons form around Purkinje cell bodies (Fig. 10). The primary axonal projection of basket cell axons is in the anterior-posterior

direction in the folia (i.e., orthogonal to the plane of parallel fiber projections), from which about 10 collateral branches descend to innervate the cell bodies of Purkinje cells, contacting about a dozen Purkinje cells in the transverse plane of the folia (but not innervating the Purkinje cell closest to it). Additional collateral branches from the primary axon travel perpendicularly in the longitudinal plane of the folia, contacting about three to six rows of adjacent Purkinje cells on each side of the primary axon. It is estimated that this organization allows each basket cell to contact a patch of 100 to 200 Purkinje cells. The functional output of basket cells is inhibitory, and the available evidence indicates that GABA is the inhibitory neurotransmitter.

3.2.4. Stellate Cells

Stellate cells derive their name from the star-shaped appearance of their dendrites. They are usually located in the outer two-thirds of the molecular layer. Their axons synapse only on Purkinje cell dendritic shafts (Fig. 9 and Fig. 10). Stellate cells are functionally similar to the basket cells because they receive the same inputs and, like the basket cells, they function to inhibit Purkinje cell firing. However, because they synapse farther from the Purkinje cell body than do the basket cells, the inhibitory influence of stellate cells is less than that of basket cells. It is believed that stellate cells use GABA as their neurotransmitter.

3.2.5. Golgi Cells

Golgi cells are large neurons scattered in the superficial part of the granular layer immediately deep to the Purkinje cell bodies. Golgi cells arise from precursors that leave the ventricular germinal zone but that continue to divide as they migrate through the white matter toward the cerebellar cortex. Estimates of the size of the Golgi cell population range from 1:10 Golgi cells to Purkinje cells (Eccles et al., 1967) to a near 1:1 ratio (Ito, 1984). Golgi cell bodies are approximately the same size as those of Purkinje cells. Although Golgi cell dendrites are not as elaborate as those of Purkinje cells, the region containing their dendrites extends over a larger expanse of the cerebellar cortex (Fig. 9 and Fig. 10). Unlike the dendrites of Purkinje cells, Golgi cell dendrites are not compressed into the sagittal plane of the folia, but instead project both to the molecular and granular layers. The dendrites that enter the molecular layer overlap the dendritic fields of three Purkinje cells in each plane, so that they act as

a central point in a functional hexagon that influence about 10 Purkinje cells. Golgi cells receive inputs from mossy fibers. The dendrites that stay in the granular layer contribute to a complex synaptic structure called a *glomerulus* that includes mossy fiber terminals and granule cell dendrites. Golgi cell axons are conspicuous in appearance. They are short, branch profusely, and also contribute to the formation of the glomerulus. The output from Golgi cells is inhibitory, with GABA as the probable neurotransmitter.

3.2.6. Unipolar Brush Cells and Lugano Cells Are Modulatory Interneurons

Unipolar brush cells (UBCs) are a recently recognized class of excitatory, glutamatergic interneurons that are found most prominently in the granular layer of the vestibulocerebellum (Nunzi et al., 2001). UBCs are generated shortly after Purkinje cells during the same period as Golgi cells (Sekerkova et al., 2004). UBCs receive extensive excitatory input from extrinsic mossy fibers and project as an intrinsic source of axons within the granular layer to excite nearby granule cells. They likely function to amplify or extend the local effects of mossy fiber input. Lugano cells are inhibitory neurons found in the granular layer near the Purkinje cell layer (Sahin and Hockfield, 1990) and are roughly as numerous as Golgi cells. Lugano cells are activated by serotonergic inputs and their axons innervate Golgi cells in the granular layer, where they are inhibitory (with GABA as their likely neurotransmitter). The axons of up to 10 Lugano cells may converge onto a single Golgi cell, and an individual Lugano cell may divergently inhibit up to 150 Golgi cells, suggesting that they may regulate synchronous activity of Golgi cells.

3.3. The Intracortical Circuitry of the Cerebellum Produces a Modulated Inhibitory Output

Based on data from a variety of experimental sources, much of the functional circuitry of the cerebellar cortex has been determined. The connections between mossy fibers and granule cells, mossy fibers and UBCs, UBCs and granule cells, granule cells and Purkinje cells, and climbing fibers and Purkinje cells all are excitatory (Fig. 7 and Fig. 10). However, the excitation of Purkinje cells is modulated by several feedback circuits that inhibit Purkinje cell activity and therefore suppress transmission from the cerebellar cortex to the deep nuclei. The excitatory and inhibitory zones of influence on the Purkinje cells

are organized in perpendicular directions in a given patch of cerebellar cortex, with a striking geometric regularity that is preserved across all vertebrates. Purkinje cells that are aligned in the mediolateral axis, such that they share excitatory input from the same “beam” of parallel fibers, form a network of neurons receiving common excitatory input (“on-beam parallel fiber network”). Concurrent activation of basket and stellate cells associated with the on-beam activity is thought to inhibit adjacent Purkinje cells in the anterior and posterior directions (orthogonal to the “on-beam” direction), thus suppressing Purkinje cell activity in the adjacent “off-beam” parallel fiber network. Thus, the inhibitory circuits usually limit both the area of the cerebellar cortex that is stimulated and the degree of excitation produced by an incoming signal. In this fashion, Purkinje cell output to the deep cerebellar nuclei is a finely calibrated inhibitory signal both topographically and temporally.

4. THE INPUT AND OUTPUT SYSTEMS OF THE CEREBELLUM

4.1. There Are Three Basic Categories of Input Fibers to the Cerebellum

Inputs to the cerebellum far outnumber its outputs (about 40:1 in humans), and inputs are directed mainly to the cerebellar cortex (Fig. 7). The inputs classically have been combined into two major pathways, *climbing fibers* (olivocerebellar tract) and *mossy fibers* (all other inputs). A third, small group of *aminergic fibers* also reaches the cerebellar cortex. All of the afferents to the cerebellum arrive through one of the three cerebellar peduncles, but primarily through the inferior and middle peduncles (Table 1).

4.1.1. Climbing Fibers

Classically, climbing fibers have been described as arising from neurons in the inferior olive, which project to the contralateral cerebellar cortex (Fig. 7, Fig. 10; *see also* Fig. 13), and the vast majority clearly do arise from the inferior olive and accessory olfactory nuclei. Climbing fibers are named for the manner in which they ascend into the molecular layer to entwine themselves, in ivy fashion, around the dendritic trees of target Purkinje cells. Olivary neurons give rise to axons that project contralaterally, enter the cerebellum, and give off collaterals to the deep cerebellar nuclei before entering cortex, where they branch in the sagittal plane to innervate several Purkinje cells. Early in development, a Purkinje cell may be innervated by multiple climbing fibers but, as development progresses, all but

one are eliminated, leaving the Purkinje cell innervated by only one climbing fiber. Each climbing fiber makes hundreds of synaptic contacts with the dendrites of the Purkinje cell, and activation of the climbing fiber can generate very large excitatory postsynaptic potentials that stimulate complex-spike action potentials that include superimposed calcium spikes that invade the dendrites. This anatomic arrangement provides for powerful, precise activation of small sets of Purkinje cells, with no convergence and limited divergence of the climbing fiber input. Although climbing fibers exert a powerful excitatory influence on individual Purkinje cells, they also provide an excitatory, albeit weaker, input to the Golgi cells and basket and stellate cells and to the deep cerebellar nuclei through collateral branches. The major neurotransmitter of olfactory climbing fibers is glutamate, and they also express corticotropin-releasing factor (CRF) as a modulatory neuropeptide (Fig. 10). Interestingly, climbing fibers also appear to serve a trophic function during development. They also are critical for inducing synaptic long-term depression (LTD) in the cerebellar cortex (Ito, 1984; Ito, 2006; Jorntell and Hansel, 2006). The role of climbing fibers in LTD and learning and neuroplasticity is supported by two important observations. Concurrent synaptic activation of target Purkinje cells by climbing fibers and parallel fibers can induce LTD, a form of enduring modification of the efficacy of the parallel fiber synapses that suppresses their subsequent excitatory control over the target Purkinje cells. LTD has been implicated as a mechanism of motor learning and adaptive changes in cerebellum (Ito, 2006; Jorntell and Hansel, 2006). Because climbing fibers have been shown to carry information regarding “errors” in the functional outcome of neural systems controlling motor function, activating processes of cerebellar plasticity such as LTD is a candidate mechanism of corrective changes during adaptation or learning of motor responses.

4.1.2. Mossy Fibers

The mossy fiber projections to the cerebellum are extensive and include all of the cerebellar afferents except the climbing fibers and the aminergic projections (Fig. 7 and Fig. 10). They enter the cerebellum through all three peduncles. Myelinated mossy fiber axons bifurcate many times in the white matter then lose their myelination as each branch gives off numerous collaterals in the granular layer. Large clump-like swellings, called *rosettes*, are specialized synaptic endings that occur repeatedly along the course of the axon branches. Each mossy fiber rosette forms the nucleus of a complicated structure called a *glomerulus*.

(Fig. 10). A glomerulus is a large synaptic complex composed of one mossy fiber rosette, dendritic contacts from many granule cells, the proximal portions of Golgi cell dendrites, and terminal branches of Golgi cell axons. The entire structure is encapsulated in a glial sheath. Each mossy fiber synapses with several hundred granule cells, and each granule cell receives mossy fiber input from several different mossy fibers, resulting in considerable divergence and convergence of input. Mossy fibers also send collaterals to the deep cerebellar nuclei. In contrast with climbing fibers, mossy fibers activate Purkinje cells indirectly by activating granule cells. In view of the wide variety of their origins, it is not surprising that their neurotransmitters are not fully characterized. However, considering that all mossy fibers are believed to be excitatory, glutamate is the neurotransmitter for many mossy fibers, and other postulated neurotransmitters include acetylcholine and the neuropeptides substance P and somatostatin (Ito, 2006).

4.1.3. Aminergic Fibers

Experimental studies with animals have identified amine-containing projections to the cerebellum (Fig. 10) (Schweighofer et al., 2004). A projection of noradrenergic fibers from the locus coeruleus (A_6 cell group) travels through the superior cerebellar peduncle to synapse with Purkinje cell dendrites in all parts of the cerebellar cortex. Dopamine-containing fibers from the $A10$ cell group of the ventral mesencephalic tegmentum project to Purkinje and granular cell layers and also to the interposed and dentate nuclei. Another input from cell groups B_5 and B_6 in the raphe nuclei in the brain stem is reported to enter the cerebellum through the middle cerebellar peduncle to provide serotonergic fibers to the granular and molecular layers. One target of the serotonergic input is the Lugano cells.

4.2. There Are Three Primary Sources of Cerebellar Inputs

Although information reaches the cerebellum from a variety of sources (Table 1), the three major sources of afferents are the spinal cord, vestibular system, and the cerebral cortex via brainstem nuclei (Fig. 11, Fig. 12, and Fig. 13).

4.2.1. Inputs from the Spinal Cord

Information from the spinal cord reaches the cerebellum through the ventral spinocerebellar tract, the dorsal spinocerebellar tract, and the cuneocerebellar tract (Fig. 12). A comparable projection from the head/neck region comes from the trigeminal system.

They provide information on the position and status of muscles, tendons, and joints. The ventral spinocerebellar tract transmits proprioceptive information from all parts of the trunk and limbs, ascending on both sides in the white matter of the spinal cord. The contralateral fibers then recross to the ipsilateral side, and all of them enter the cerebellum through the superior cerebellar peduncle, terminating as mossy fibers. The dorsal spinocerebellar tract conveys proprioceptive information from the lower trunk and lower limbs. This projection of mossy fiber inputs ascends in the spinal cord on the ipsilateral side and enters the cerebellum through the inferior cerebellar peduncle. The cuneocerebellar tract transmits proprioceptive information from the upper trunk and upper limbs. Axons arising from the lateral (accessory) cuneate nucleus in the medulla oblongata enter the ipsilateral cerebellum through the inferior cerebellar peduncle to terminate as mossy fibers. It should be noted that all spinal cord afferent pathways to the cerebellum arise and terminate on the *ipsilateral* side.

Data from experimental studies have demonstrated that the inputs from proprioceptive and tactile stimuli to the cerebellar cortex are organized in two somatotopic maps (homunculi), one centered in the vermis of the anterior lobe and the other (actually a double one consisting of bilateral mirror images) in the posterior lobe. These cerebellar homunculi are considerably less precise than those in the cerebral cortex.

4.2.2. Inputs from the Vestibular System

Primary fibers from the vestibular labyrinth travel via cranial nerve VIII to reach the ipsilateral cerebellum through the juxtarestiform body (Fig. 11). A much larger bundle of fibers is directed to the vestibular nuclei in the brain stem, where it is subsequently relayed through the juxtarestiform body to terminate bilaterally in the flocculonodular lobe and most of the remaining vermis as mossy fibers. These vestibular fibers provide important information pertaining to equilibrium.

4.2.3. Inputs from the Cerebral Cortex

Information from multiple regions of the cerebral cortex reaches the cerebellum indirectly from three different pathways: the (cortico) pontocerebellar tract, the (cortico) olivocerebellar tract, and the (cortico) reticulocerebellar pathway (Fig. 13).

This information, which is destined for the cerebellum, originates from cells in various regions of the cerebral cortex and synapses in the pontine nuclei, the inferior olive nuclei, and the reticular formation.

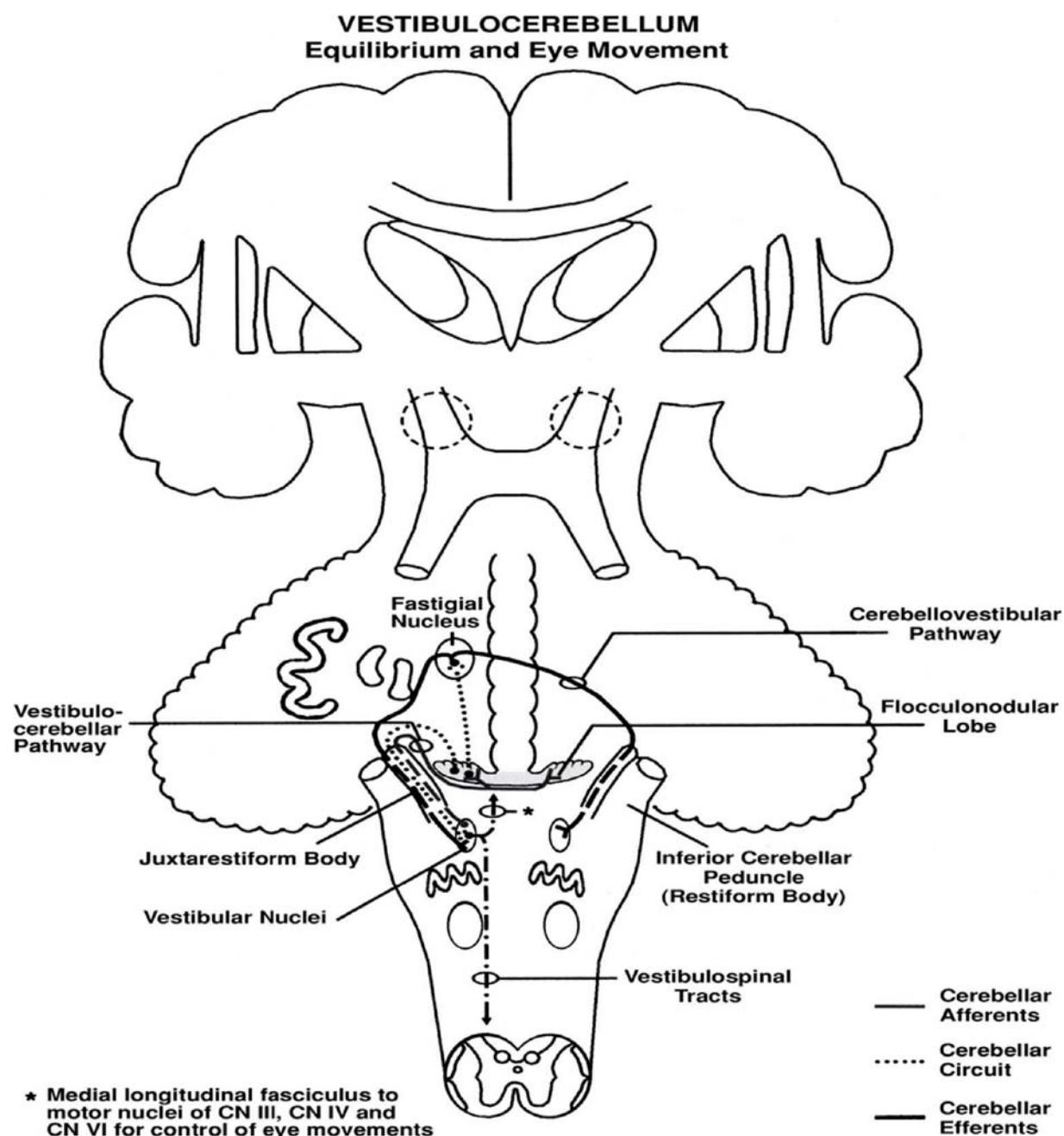


Fig. 11. Schematic diagram of the brain and spinal cord, showing the primary afferent and efferent projections of the vestibulocerebellum.

The (cortico) pontocerebellar pathway continues from neurons in the pontine nuclei that give rise to fibers that cross the midline and enter the cerebellum on the contralateral side through the middle cerebellar peduncle. This huge fiber tract projects to the cerebellum as mossy fibers. The olivocerebellar pathway relays information from the cerebral cortex that projects to the inferior olive. Inferior olivary fibers cross the

midline to enter the cerebellum through the contralateral inferior cerebellar peduncle, where they terminate directly on Purkinje cell dendrites as climbing fibers. The reticulocerebellar pathway also relays information from the cerebral cortex. Neurons in the brain-stem RF give rise to fibers that enter the cerebellum on the ipsilateral side through the inferior cerebellar peduncle to end as mossy fibers.

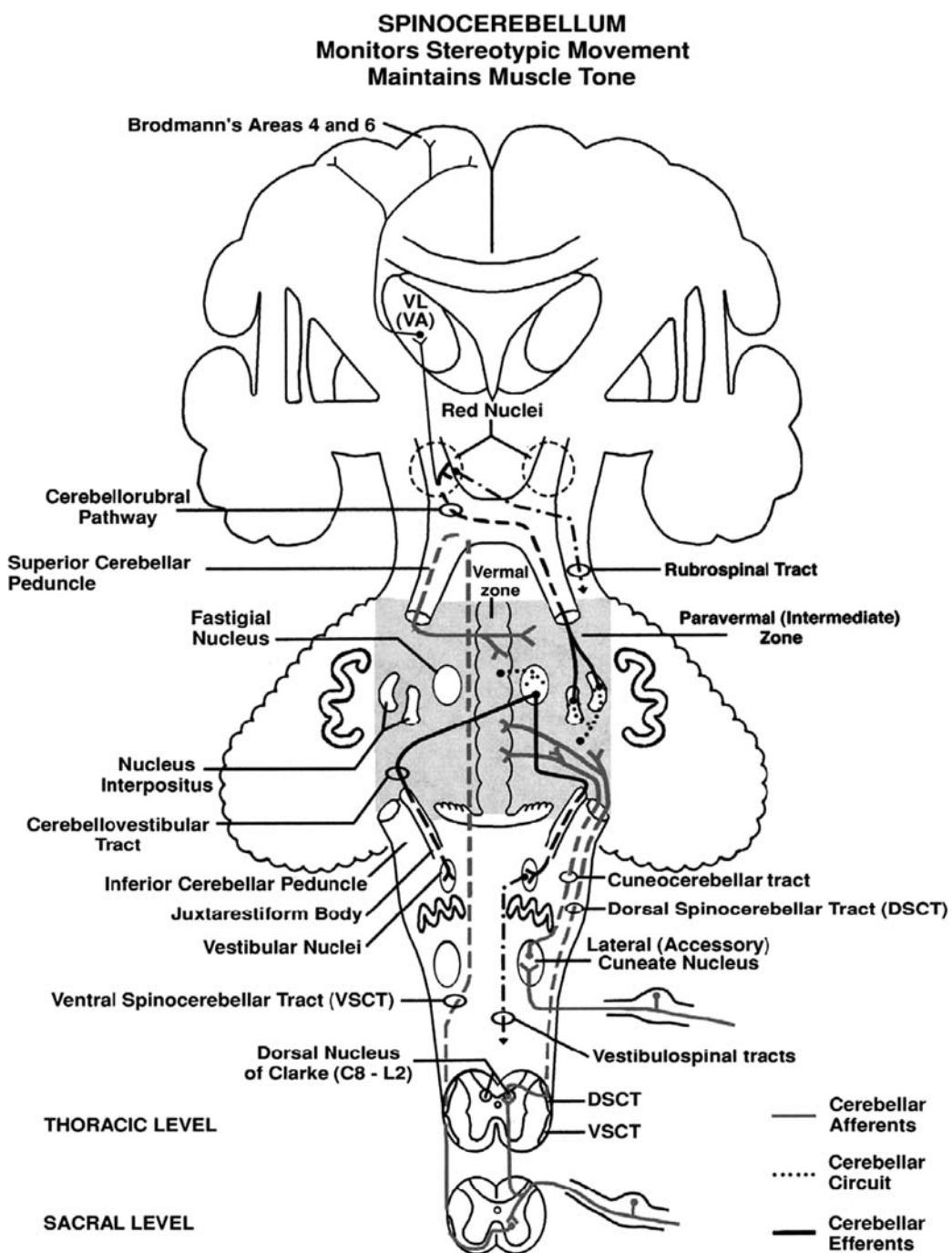


Fig. 12. Schematic diagram of the brain and spinal cord, showing the primary afferent and efferent projections of the spinocerebellum.

4.3. The Outputs from the Cerebellum Are Less Complex than the Inputs

There are many fewer outputs from the cerebellum than inputs. The sole output from the entire cerebellar cortex is from the axons of Purkinje cells. However, information from most of the Purkinje cells is relayed

through neurons in the deep cerebellar nuclei before exiting the cerebellum (Fig. 11, Fig. 12, Fig. 13, and Fig. 14). All outputs from the cerebellum exit through either the superior or inferior cerebellar peduncles.

The majority of outputs from the cerebellum exit through the superior cerebellar peduncle. The small

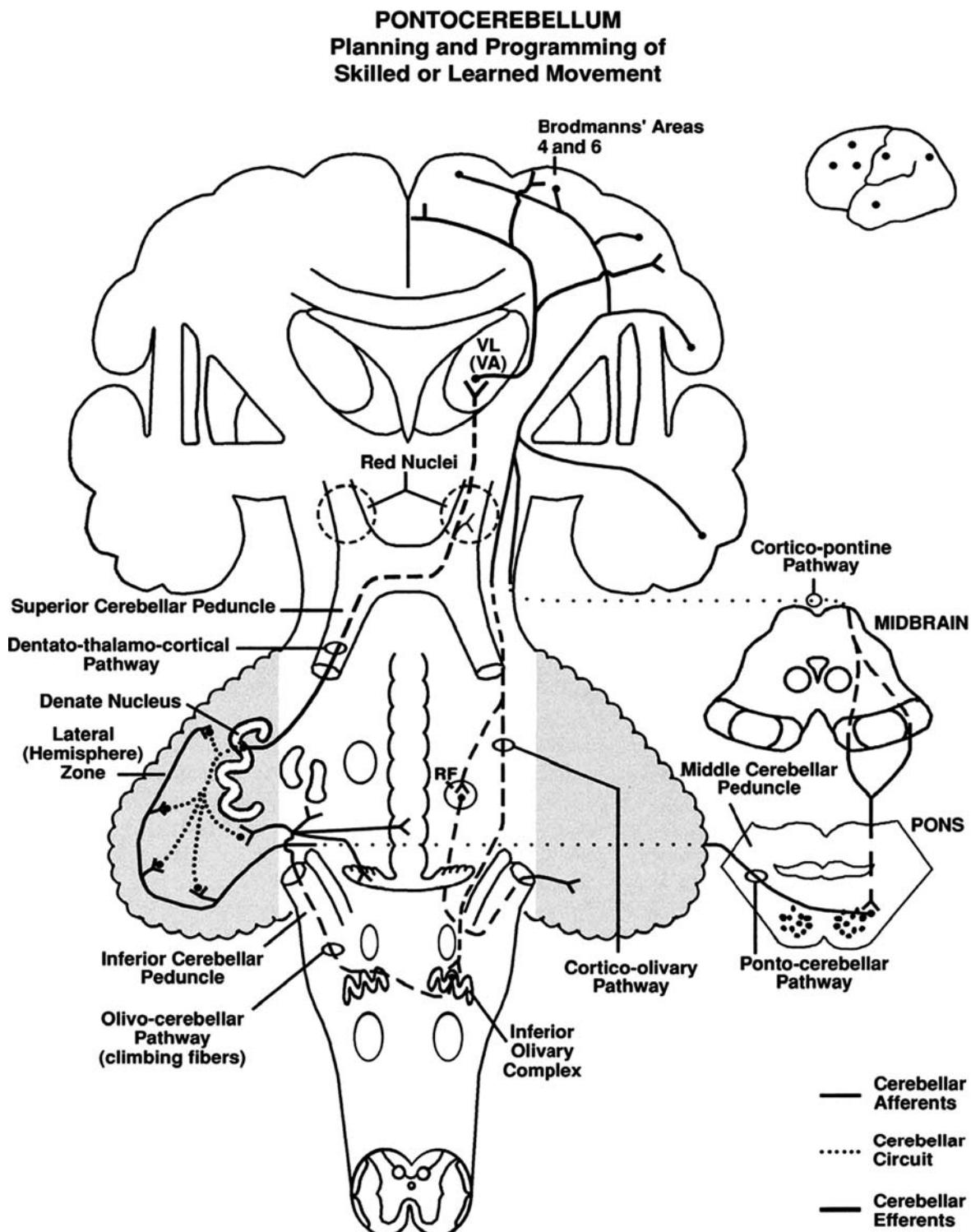


Fig. 13. Schematic diagram of the brain and spinal cord, showing the primary afferent and efferent projections of the pontocerebellum.

projection of Purkinje cell axons that do leave the cerebellum directly travel through the juxtarestiform body of the inferior cerebellar peduncle and synapse in the vestibular nuclei. The output from the other

Purkinje cells project to the deep cerebellar nuclei in a specific medial-to-lateral pattern, and the principal efferents from the cerebellum originate from neurons in these deep nuclei (Fig. 11, Fig. 12, Fig. 13, and

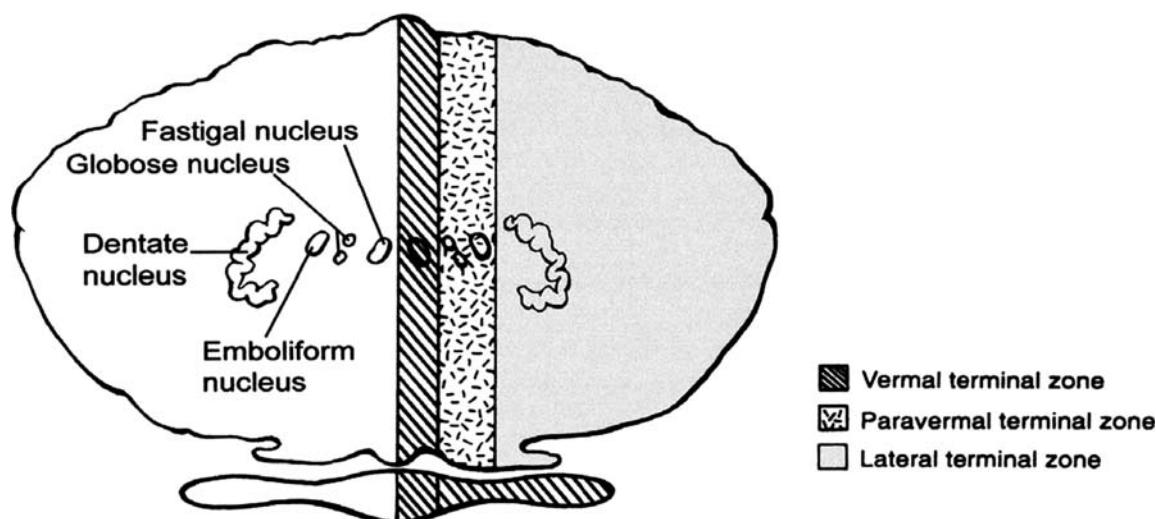


Fig. 14. Diagram illustrating the zones of organization of the cerebellum based on the longitudinal termination of inputs to the cerebellar cortex and their projections to the deep cerebellar nuclei. Compare with Fig. 4.

Fig. 14). Purkinje cells in the vermis project to the fastigial nucleus, Purkinje cells in the intermediate zone project to the globose and emboliform nuclei, and those in the lateral cerebellar hemispheres project to the dentate nucleus. Some Purkinje cells from the flocculus and nodulus project to both the fastigial and dentate nuclei as well as to the vestibular nuclei.

The outputs from the fastigial nucleus project mainly to the brain stem, where they synapse in the vestibular nuclei bilaterally, and in the reticular formation, where the projection is primarily contralateral. The ipsilateral projections from the fastigial nucleus travel through the juxtarestiform body. The fibers destined for contralateral regions cross the midline while still within the cerebellum, hook around the superior cerebellar peduncle (hence the name *hook bundle* or *uncinate fasciculus*), and then exit through the juxtarestiform body on the contralateral side. Small contralateral projections also reach the cervical spinal cord and the thalamus (Fig. 11).

The largest output from the cerebellum consists of axons passing through the superior cerebellar peduncle (brachium conjunctivum) (Fig. 13). The cells of origin are located in the globose, emboliform, and dentate nuclei. Some of these cells project axons to the inferior olive and RF (the descending limb of the superior cerebellar peduncle). However, the vast majority of the fibers in this peduncle project rostrally to cross to the contralateral side in the midbrain at the level of the inferior colliculus, as the decussation of the superior cerebellar peduncle. Many of the axons that originate in the emboliform and globose nuclei, and a few fibers from the dentate nucleus, synapse in the contralateral

red nucleus. The majority of the remaining axons from the dentate nucleus (the *dentatorubrothalamic tract*) continue on to the contralateral thalamus, where they terminate primarily in the VL nucleus, with a smaller contingent terminating in the VA nucleus.

In summary, with the exception of a small bundle of Purkinje cell axons that project directly to the vestibular nuclei in the brain stem, all outputs of the cerebellum consist of axons with cell bodies located in the deep cerebellar nuclei. Each fastigial nucleus receives input from the vermis and projects bilaterally to the vestibular nuclei. The globose and emboliform nuclei on each side receive input from the ipsilateral paravermis and project mainly to the contralateral red nucleus. Each dentate nucleus receives input from the ipsilateral lateral hemisphere and projects primarily to the VL of the contralateral thalamus (Fig. 11, Fig. 12, and Fig. 13).

5. THE CEREBELLUM AND MOTOR LEARNING

The cerebellum appears to play an important role in learning motor skills involving acquisition of accurate, rapid, and agile complex voluntary movements, such as playing a musical instrument or executing a picture-perfect jump shot. With repetitions of the movements during acquisition of motor skills, “errors” identified by activation of specific climbing fibers can induce enduring changes in synaptic activity in the cerebellar cortex that lead to anticipatory correction of subsequent movements. Though multiple forms of activity-dependent plasticity have been described in

the cerebellum, the form most prominently implicated is long-term depression (LTD) of the efficacy of parallel fiber-Purkinje cell synapses (Ito, 2006; Jorntell and Hansel, 2006). LTD occurs on those Purkinje cells that have simultaneous activation of a set of parallel fibers during the climbing fiber activation. This activity-dependent LTD in the response of the Purkinje cell to the mossy fiber input can serve as a form of memory of an incorrect outcome of a movement such that the pattern of subsequent cerebellar output to the same pattern of sensory input (via the mossy fibers) is changed.

Ito and his colleagues have extensively studied processes of neuroplasticity underlying the adaptation of eye movements of the *vestibulo-ocular reflex* (VOR), which keeps the visual image on the retina stable during periods of head rotation. When the head moves in one direction, the VOR automatically moves the eyes in the opposite direction at the same speed, so there is very little “retinal slip” of the visual image on the fovea. Information about head movement from the vestibular apparatus reaches the flocculus by way of mossy fibers, and information about retinal slip (error of intended eye movement) is conveyed by climbing fibers to the same region. Under conditions of mismatch between retinal image and direction of gaze, as when a subject puts on prism glasses that displace the retinal image by a defined angle (such that the VOR is no longer properly calibrated), adaptive changes occur over time that appear to depend on LTD in Purkinje cells of the flocculus (Ito, 2006). This synaptic plasticity results in recalibration of the VOR to the new conditions.

In humans, adaptation to prism glasses also produces a striking pattern of performance in a dart-throwing task. Once accurate baseline throwing is established without prism glasses, the subject then puts on the prism glasses and the first few subsequent throws deviate in the direction opposite to the direction of the bent light path (e.g., to the left if the light path is bent to the subject’s right). After some practice, the normal subject regains accuracy, but then when the glasses are removed, transient error deviations in the opposite direction occur (now to the right) until the subject then adapts to the absence of prism glasses. In contrast, prism adaptation in patients with cerebellar damage to the territory of the vestibulocerebellum is impaired or absent, whereas patients with damage in the distribution of the superior cerebellar artery or in motor thalamus have no adaptation impairment (Martin et al., 1996).

A second form of cerebellar-dependent learning—classic (Pavlovian) conditioning of eyeblink

responses—is one of the most extensively studied and best-understood models of mammalian associative learning in neuroscience (Woodruff-Pak and Steinmetz, 2000a; Woodruff-Pak and Steinmetz, 2000b). Eyeblink conditioning involves paired presentations of a neutral conditioned stimulus (CS; e.g., a tone) with a biologically significant unconditioned stimulus (US; e.g., an airpuff to the cornea) that elicits a reflexive eyeblink, the unconditioned response (UR). In the simplest form of classic conditioning, the CS onset occurs before (<1 s) the US and overlaps and coterminates with the US. With sufficient pairings over many trials, the subject comes to emit conditioned eyeblink responses (CRs) after presentation of the CS, in advance of the US, and these CRs are typically timed to occur just before the onset of the US. Experimental studies using lesions, reversible inactivation, neural recordings, tract-tracing, and functional neuroimaging have provided converging evidence that specific circuitry in the cerebellum and brain-stem neurons is essential for learning the CS-US association, and sites of learning-related functional and structural neuroplasticity mediating learning and performance of the conditioned eyeblink response have been identified (Thompson, 1986; Lavond et al., 1993; Thompson and Kim, 1996; Kim and Thompson, 1997; Medina et al., 2000; Christian and Thompson, 2003; Miller et al., 2003; Ohyama et al., 2003), summarized below.

The CS is projected to discrete portions of the pontine nuclei, and the mossy fiber axons from these pontine neurons project the CS to discrete regions of cerebellar cortex and to the interpositus nucleus. The US information is projected from the trigeminal system to neurons of the dorsal accessory inferior olive that give rise to the climbing fibers that distribute to discrete regions of cerebellar cortex and the interpositus nucleus. Though the cerebellum is not necessary for unconditioned eyeblinks, neuroplasticity arising from converging CS and US input projections in the cerebellar cortex and the interpositus is essential for learning and performance of conditioned eyeblinks. The efferent projections of the interpositus neurons (to red nucleus) provide the signals that reach the cranial nerve nuclei to generate the conditioned eyeblink. Neuroplasticity at the interpositus nucleus appears to be required for acquisition and performance of the eyeblink CR, as lesions or reversible inactivation completely prevent acquisition or expression of conditioning, permanently in the case of complete lesions (Lavond et al., 1993; Christian and Thompson, 2003). The interpositus appears to be both a site of memory formation and

a site of long-term memory storage of this conditioned response (Kleim et al., 2002; Christian and Thompson, 2005). The specific role of neuroplasticity at synapses converging on Purkinje neurons, including the role of LTD in Purkinje cells engaged by the conditioning stimuli, is still uncertain, but it likely controls the temporal specificity and amplitude of the CRs (Kim and Thompson, 1997; Ohyama et al., 2003). In humans, functional neuroimaging studies in normal subjects have confirmed that cerebellum is functionally activated during classic conditioning, and studies in patients with cerebellar damage or alcohol-related toxicity have confirmed deficits in eyeblink conditioning (McGlinchey-Berroth et al., 1995; Coffin et al., 2005; Gerwig et al., 2007). Eyeblink conditioning *per se* is rather artificial and of limited adaptive relevance as a behavior, but it provides a convenient and precise behavioral measure with many experimental advantages. Because nearly all somato-motor learning involving classic conditioning of discrete, skilled movements involves comparable, cerebellar-dependent processes, the insights about learning-related cerebellar neuroplasticity gained from studying eyeblink conditioning will likely have general significance to other forms of motor skill learning.

6. CEREBELLAR DYSFUNCTION

In contrast with the detailed knowledge of the uniform cortical organization of the cerebellum, surprisingly little is known about its functions in relation to other regions of the brain. Cerebellar function is best appreciated by considering the effects of lesions on specific parts of the cerebellum. With this goal in mind, two plans of functional organization have been proposed. Although they are similar, they differ somewhat in the resultant functional zones they represent. Various authors use one or the other, or both schemes, to localize cerebellar function.

Based in part on data from both clinical and experimental studies, three so-called “cerebellar syndromes” can be distinguished: (1) flocculonodular syndrome; (2) anterior lobe syndrome; and (3) neocerebellar syndrome. This distinction has been used by a number of authors to help correlate structural and functional relationships in the cerebellum and to explain deficits resulting from cerebellar lesions. The flocculonodular syndrome is characterized by problems with maintaining equilibrium, but there is no ataxia of the limbs, hypotonia, or tremor. The anterior lobe syndrome is characterized by increased postural reflexes. The neocerebellar syndrome is

distinguished by ataxia and hypotonia, producing clumsy movements. Although it is possible to demonstrate these syndromes experimentally in animals, pure cerebellar syndromes, especially the anterior lobe syndrome, are seldom manifested in humans (Brodal, 1981).

It is important to emphasize that because the local circuitry throughout the cerebellar cortex is organized as essentially identical modules, a strict localization of function based on differences in intrinsic circuitry and the types of computational functions does not exist within the cortex itself. Rather, functional localization in the cerebellum is the result of differences in the location of the termination of its afferent and efferent projections. Thus, from a clinical perspective, cerebellar function and dysfunction can best be understood by the organization of three medial-to-lateral longitudinal zones corresponding with the terminal fields of major inputs to the cerebellar cortex and the medial-to-lateral organization of projections to the deep nuclei (Fig. 9). These three longitudinal (sagittal) bands are the vermal, paravermal, and lateral zones. This organization represents a crude parcellation of afferent termination zones in the cerebellar cortex, but it should be recognized that it has considerable overlap. The cerebellar cortex continues the longitudinal organization by projecting from the three longitudinal cortical zones to corresponding deep cerebellar nuclei (Fig. 14).

Damage to the cerebellum or its associated afferent and efferent systems produces distinctive symptoms and signs, *usually on the same side of the body as the lesion*. Not surprisingly, lesions of deep nuclei and/or superior cerebellar peduncle (e.g., lesions that interrupt cerebellar outflow) produce more severe signs than do lesions restricted to parts of the cerebellar cortex. Signs of cerebellar dysfunction in humans are usually the result of lesions that involve more than one specific region of the cerebellum. They manifest themselves as deficits in somatic motor control, with the most common symptoms related to disturbances of gait. For organizational purposes, it is worthwhile to consider separately the results of damage to the vermal, paravermal, and lateral zones. There have been a number of excellent descriptions and reviews of the disorders of cerebellar function (Holmes, 1939; Dow and Moruzzi, 1958; Gilman et al., 1981), to which interested readers are referred.

6.1. Vermal Zone Cerebellar Damage

The vermal or midline longitudinal zone consists of the vermis, the flocculi, the fastigial nuclei, and related

input and output fibers. The principal afferents to this zone are from the vestibular organ and nuclei, from the trunk and neck via the spinal cord, and from the reticular formation. Some primary inputs from the vestibular part of the vestibulocochlear nerve (cranial nerve VIII) enter the cerebellum as part of the juxtarestiform body and terminate in the flocculus and nodulus—with a small projection to the uvula and lingula of the vermis—as well as sending collaterals to the fastigial nucleus. A larger projection of secondary fibers from the vestibular nuclei also project to most of the vermal zone. Proprioceptive information from the head, particularly from the temporomandibular joint (TMJ) and muscles of mastication, are transmitted through the trigeminocerebellar tract, which originates from the mesencephalic trigeminal nucleus, and projects to parts of the vermis, paravermis, and flocculus via the restiform body.

Functionally, the vermal zone is associated with posture as controlled by trunk (axial) musculature and proximal limb muscles, head position, extraocular movements, equilibrium, and locomotion (Thach and Bastian, 2004). Clinical signs resulting from midline cerebellar damage produce disorders of posture and gait, head positions, and eye movements (e.g., nystagmus). Because of its connections with the vestibular system, damage to the flocculus, nodulus, and uvula result in a pronounced loss of equilibrium, including truncal ataxia (swaying while standing or staggering while walking with a tendency to fall, usually backwards), and a compensatory, wide-based stance and/or gait. There is an inability to incorporate vestibular information with body and eye movements. When damage is restricted to the vermal zone, these deficits and truncal asynergia are usually present without the disruption of function in individual limbs when tested separately (Gilman, 1986). Midline cerebellar lesions may also result in varying levels of tremor, known as *titubation*, involving the head and/or trunk.

A clinical picture corresponding with the vermal or flocculonodular syndrome seen in children between 5 and 10 years of age occurs as a result of a specific type of tumor known as a medulloblastoma. It usually occurs in the nodulus of the vermis, is characterized by unsteady gait and frequent unexplained falls, and may be accompanied by nystagmus. Medulloblastoma is a neoplasm that is derived from persistent clumps of germinal cells from the external granular layer. This germinal field normally dissipates after about 2 years of age, which helps to explain why this tumor is the most common type of central nervous system tumor in children, but never occurs in adults.

6.2. Paravermal Zone Cerebellar Damage

The functional paravermal (or intermediate) zone consists of the cerebellar cortex just lateral to the vermis, together with the emboliform and globose nuclei and interconnected inputs and outputs. The principal source of information to the paravermal zone is the limbs, but afferents are from a variety of sources from spinal cord, brain stem, and cerebral cortex. Outputs project to both rostral and caudal portions of the nervous system. Damage restricted solely to the paravermal zone is found only in experimental animals. In humans, lesions of this region are typically an extension of existing lesions in the vermal or lateral regions of the cerebellum. Functionally, this region seems to be involved in the modulation of velocity, force, and the pattern of muscle movement and changes in muscle tone (either hypertonia or hypotonia).

6.3. Lateral Zone Cerebellar Damage

The large lateral zone consists of most of the cerebellar hemispheres (including much of the anterior lobe), the dentate nuclei, and related inputs and outputs. Inputs are from numerous sources, but most important is the projection from the cerebral cortex that is relayed through the pons. Outputs are to the brain stem and thalamus. This region is involved in the planning of voluntary movements in conjunction with the cerebral cortex. Damage to the large lateral hemispheric regions affects the initiation, timing, and coordination of volitional movements. The most common type of cerebellar disease involves the hemispheres or some part of their efferent projections. Damage to the lateral zone produces movement disorders of the limbs as well as difficulty in posture and gait. If the lesion is unilateral, the signs are ipsilateral; the patient tends to stagger and deviate to the affected side.

There are a number of common disturbances associated with damage to the lateral cerebellar zone. A lesion of this region can result in any combination of the following signs and symptoms: *Ataxia* is a general term for clumsiness or disturbances in coordination of motor activity related to voluntary movement. *Asynergia*, or limb ataxia, is the reduced ability to execute smooth, coordinated sequential movements. *Hypotonia* is a reduced resistance to passive movement caused by a loss of cerebellar influence. *Asthenia* is an increased propensity for muscle fatigue, which is often associated with hypotonia. *Intention tremor* is an oscillating movement of a limb, which is present only during limb movement. This tremor is particularly pronounced toward the end of a given movement, such as reaching for an object. “Intention” tremor is

in contrast with the Parkinson “resting” tremor, which is manifested while the extremity is at rest. *Adiadochokinesia* (*dysadiadochokinesia*) refers to uncoordinated, irregular movements that occur during attempted rapid alternating movements, such as pronating and supinating the forearm. In *dysmetria*, the judgment of distance is impaired. This is evident from the inaccurate control of the range and direction of movement, resulting in either undershoot or overshoot of the desired point (“past-pointing”). A related sign is known as “impaired check and rebound.” This can be elicited by asking the patient to lean their chest firmly against the examiner’s hands, then abruptly removing the examiner’s hand. In a patient with normal cerebellar function, their forward movement is quickly checked. The patient with a lateral cerebellar lesion will continue forward, with the patient typically losing their balance or rocking back and forth. There also can be ocular signs, especially *nystagmus*, which is of greatest amplitude when looking to the same side as the lesion. *Decomposition of movement* is a term given to the breaking down of a complex movement into its various components, which appear jerky and uncoordinated, rather than appearing as a smooth, flowing movement. *Dysarthria* refers to disorders in the mechanical component of the articulation of speech resulting from ataxia of the muscles controlling generation of speech and results in a typical pattern involving explosive, halting speech with slurred or garbled words and difficulty in modulating volume.

6.4. Cerebellar Damage and Nonmotor Functions

Historically, the cerebellum has been considered to be involved in the generation or regulation of movement. However, with more advanced neuroanatomic methods to trace transneuronal projections to and from the cerebellum, together with more sophisticated neuropsychological and functional neuroimaging approaches of modern experimental cognitive neuroscience, substantial evidence indicates that the cerebellum makes important contributions to perceptual, cognitive, and affective functions.

Damage to the cerebellum interferes with the timing of discontinuous movements (Spencer et al., 2003), and it appears to do so by disrupting the ability to properly process temporal information about sensory and motor events (the temporal representation of events) (Spencer et al., 2005). This impairment may also account for deficits in judgments of time in mental or perceptual tasks (Ivry and Spencer, 2004).

Additional neuropsychological evidence indicates that the cerebellum is involved in the early development and retention of cognitive skills in humans (Riva and Giorgi, 2000; Allin et al., 2001).

A pattern of effects on cognitive and emotional processes has been described in children and adults with cerebellar lesions of various etiology (stroke, infection, tumor and surgical resection, trauma, degeneration, developmental hypoplasia, toxicity, complications of preterm delivery), and these may be evident even without motor symptoms and may include psychiatric manifestations (Schmahmann and Sherman, 1998; Schmahmann, 2004). Lesions that involve the vermis can produce effects on affective state and emotional processing, including altered autonomic regulation, blunted affect, behavioral disinhibition, and personality changes, perhaps reflecting disrupted functional connections with the hypothalamus and limbic cortex. When lesions involve the posterior lobes of the hemispheres, impairments have been described for executive functions such as planning or correcting sequences of action during goal-directed behavior, shifting to new strategies when conditions change, and maintaining information in working memory, especially verbal information (Bellebaum and Daum, 2007). Cerebellar damage has been historically shown to produce speech motor disorders (ataxic dysarthria), but damage to the neocerebellum is also now recognized to produce other deficits in linguistic processing (Ackermann et al., 2007), including agrammatism and transient mutism, particularly when it involves the right cerebellar hemisphere. Deficits in noun-verb word associations have been reported in some patients with right cerebellar hemisphere damage.

Among the specific cognitive functions suggested for the cerebellum, converging evidence now provides compelling support for a significant role in verbal working memory. Functional neuroimaging studies consistently demonstrate activation of the right cerebellum, in conjunction with left frontal opercular region (Broca’s area), during verbal working memory tasks, during which verbal information must be encoded and actively maintained (covertly rehearsed) to successfully perform the task (Chen and Desmond, 2005). Damage to the cerebellum produced relatively selective deficits in verbal working memory (Ravizza et al., 2006), and short-term disruption of cerebellar activity with transcranial magnetic stimulation interfered with verbal working memory more than with a motor control task (Desmond et al., 2005). Cortico-ponto-cerebellar circuits between the frontal lobe of the language-dominant hemisphere and the contralateral cerebellar hemisphere likely form the

functional loops that mediate verbal working memory, for which encoding and maintenance functions may be spatially segregated.

6.5. General Concepts Related to Cerebellar Damage

Relatively small lesions to the cerebellar cortex produce few discernible deficits. Even the diagnosis of substantial cerebellar damage is often difficult because the effects are often transient, suggesting that recovery due to functional plasticity can occur. An excellent example of this recovery was documented in a case report of an 18-year-old male who underwent resection of a right cerebellar hemisphere mass. Shortly after the surgery, significant and specific deficits were shown in verbal working memory, but these resolved by 5 months postsurgery (Silveri et al., 1998). Generally, the onset and spread of pathologic processes is more rapid in older patients, typically resulting in better-defined and more permanent symptoms in this age group. In some case reports, functional compensation in response to cerebellar damage in fetal and neonatal life results in essentially no major signs of cerebellar damage, even in cases where an entire cerebellar hemisphere is completely absent (Brodal, 1981).

The cerebellum is vulnerable to damage from a wide variety of sources, including developmental defects, degenerative diseases (both hereditary and nonhereditary), infectious processes, chronic alcoholism, toxic and metabolic effects (including hypoxia), thrombosis of the cerebellar arteries, trauma, and tumors. Damage can occur from either direct or indirect sources. Tonsillar herniation, for instance, causes the cerebellar tonsils to be squeezed out of the base of the skull through the foramen magnum as a result of a tumor or hemorrhaging (Adams et al., 1984). In recent years, considerable experimental data indicate that severe malnutrition during development (Dobbing, 1981) and fetal alcohol exposure (West, 1986; Green, 2004) both produce permanent structural damage to the cerebellum with functional consequences.

Lesions restricted to the vermis produce disturbances of the trunk (stance and gait) and typically become permanent disabilities. Lesions of the lateral parts of the cerebellar hemispheres produce disturbances chiefly related to voluntary, skilled movements of the limbs and fingers but may not show enduring motor abnormalities. However, one of the most consistent effects of damage to the right cerebellar hemisphere is impairment in verbal working memory. The most prevalent clinical

symptoms of cerebellar damage are associated with disruptions of standing and walking. The identification of the loci of damage based on the analysis of cerebellar symptoms is often difficult, as more than one functional zone is usually affected. Symptoms usually dissipate with time, particularly when the damage occurs in childhood or develops slowly, over a long period of time. Cerebellar damage can have many etiologies. Secondary effects such as pressure, tissue dislocation, or vascular changes caused by associated circulatory disturbances also can produce serious cerebellar damage (Brodal, 1981).

To summarize, there are five important concepts to keep in mind when considering lesions of the cerebellum. These concepts are as follows:

1. Lesions of the cerebellum or its afferent or efferent pathways may disrupt normal coordinated movements but will not cause paralysis.
2. Because of its internal and external anatomic connections, each cerebellar hemisphere exerts its influence on the muscles of the ipsilateral side of the body.
3. The flocculonodular lobe influences the axial musculature bilaterally.
4. Damage to the lateral hemispheres can manifest as deficits in skilled movement and deficits in cognitive skills or temporal processing, but the effects of cerebellar damage are typically much less severe than is damage to functionally related neocortical regions.
5. Because of the convergence of the output of the cerebellar hemispheres to the deep cerebellar nuclei and the further subsequent compaction of efferents through the superior cerebellar peduncle, lesions of the efferent pathway (deep cerebellar nuclei and/or superior cerebellar peduncles) produce more profound and permanent deficits than do lesions of the afferent pathways or cerebellar cortex.

7. SUMMARY

For neuroscientists, the cerebellum has been a favorite target for experimental analysis, in part because of the geometric simplicity of its neuronal architecture and patterns of inputs and outputs, its distinct developmental patterning, and its remarkable capacity for physiologic and synaptic neuroplasticity to alter the function of specific neuronal circuits. Despite the uniformity of the modular structural organization across the cerebellum, and recent advances in understanding the connectional networks that define

the function of the modular zones, remarkably little is known about the precise operations or even the essential computational functions carried out by the cerebellum. The organization of the neuronal machinery of the cerebellum, considering inputs, outputs, and intracerebellar connections, likely defines its general function of comparing cortical signals of intended movements or cognitive plans with sensory signals reporting on events associated with the actual movement or outcomes to generate feed-forward signals that can modify subsequent actions or strategies. The cerebellum receives an error signal through the climbing fibers about outcomes of the intended movement that can activate mechanisms of neuroplasticity and learning to modify the next occurrence of the movement and tailor it to the appropriate environmental and sensory signals.

A wide variety of experimental studies have generated convincing data demonstrating that cerebellar circuitry can be altered functionally by experience and that certain types of motor learning can be prevented or altered by cerebellar lesions. Neuroplasticity in defined cerebellar circuitry in the vestibulocerebellum is essential for adaptation of the vestibulo-ocular reflex, and learning-related plasticity in circuits of the spinocerebellum are necessary for classic conditioning of discrete somato-motor responses such as conditioned eyeblinks. Likewise, the lateral cerebellar hemispheres are important for learning motor and cognitive skills acquired with extensive practice. Nevertheless, the function of the cerebellum extends beyond motor programming and motor learning in the strict sense. Advances in the past decade provide structural and functional evidence that the cerebellum is involved in higher-order functions, including encoding and maintenance of working memory, regulation of planning of goal-directed behavior and correction of that behavior when conditions change, and even regulation of emotion and mood states. More precise knowledge of the functional mapping of the cerebellum, including specific modular topography and computational processes within and between modules, combined with advances in understanding molecular mechanisms of cerebellar plasticity in relation to behavioral change, should lead to more comprehensive accounts of cerebellar functions in motor and cognitive control and learning.

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1. INTRODUCTION

The brain stem is best viewed when separated from the overlying cerebral hemispheres and cerebellum (Fig. 1, Fig. 2, and Fig. 3). The brain stem is continuous with the spinal cord at the foramen magnum of the occipital bone and with the diencephalon at the incisura of the tentorium. It consists of the midbrain, pons, and medulla, which lie in close relationship to the anterior surface of the base of the occipital bone. The vertebral and basilar arteries lie between the anterior surface of the brain stem and the occipital bone. Paired cranial nerves originate or terminate in the brain stem; the exceptions are the olfactory, optic, and accessory nerves (Fig. 1). The cerebellum lies posterior to the brain stem and is attached by three pairs of fiber bundles: the inferior, middle, and superior cerebellar peduncles (Fig. 2). The brain stem, cerebellum, roots of the cranial nerves (CNs), and

vertebro-basilar arterial system are located in the posterior cranial cavity or infratentorial space (see Chapter 2 for additional illustrations.).

2. EXTERNAL ANATOMY OF THE BRAIN STEM

2.1. Medulla

The anterior aspect of the medulla is composed of paired bundles of fibers, the *pyramids*, which are continuations of the motor pathway from the cortex to the spinal cord (Fig. 1). At the medulla-spinal cord transition, most of the motor pathway axons cross to the contralateral side forming the *motor or pyramidal decussation*; they continue, without synapse, as the *lateral corticospinal tracts* in the lateral funiculi of the spinal cord (Fig. 4A; see also Fig. 8B).

The *olives*, prominent swellings on the lateral aspect of the medulla, mark the position of the *inferior olive nuclei*, which are components of the motor system and project to the contralateral cerebellum. The *glossopharyngeal and vagus nerves*

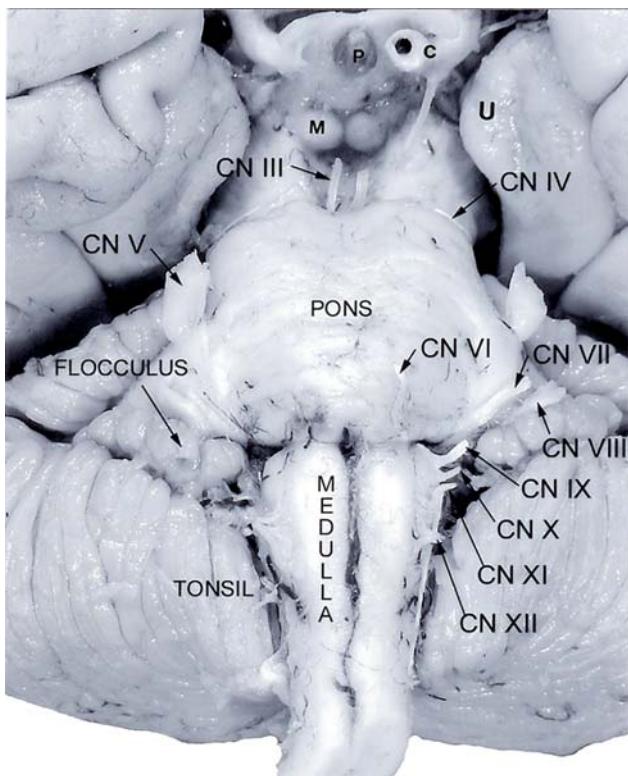


Fig. 1. Anterior view of the brain stem illustrating the cranial nerve roots. III, oculomotor nerve; IV, trochlear nerve; V, trigeminal nerve; VI, abducens nerve; VII, facial nerve; VIII, vestibulocochlear nerve; IX, glossopharyngeal nerve; X, vagus nerve; XI, accessory nerve; XII, hypoglossal nerve; C, internal carotid artery; cerebral peduncles lateral to III; M, mammillary bodies of the hypothalamus; P, pituitary gland; U, uncus of parahippocampal gyrus. The space between the cerebral peduncles is the interpeduncular fossa or cistern; the posterior aspect of this space is the posterior perforated substance (see Fig 7). Note the close relationship of the uncus to the midbrain. The medulla, pons, and flocculus of the cerebellum form the boundaries of the cerebellopontine space (angle). Note that the facial and vestibulocochlear nerves are in the cerebellopontine space. Note the close relationship between the cerebellar tonsils and the medulla.

emerge as a series of rootlets just posterior to the olives and exit the cranial cavity through the jugular foramen. Their peripheral branches provide visceral, sensory, and autonomic innervation to structures in the head and neck as well as organ systems in the thorax and abdomen. The *hypoglossal nerves* exit the medulla anterior to the olives and pass through the hypoglossal canals on course to the floor of the oral cavity. Hypoglossal nerves supply motor innervation to the extrinsic and intrinsic skeletal muscle of the tongue (Fig. 1 and Fig. 4C).

On the posterior surface of the medulla, the membranous *inferior medullary velum*, a reflection of the

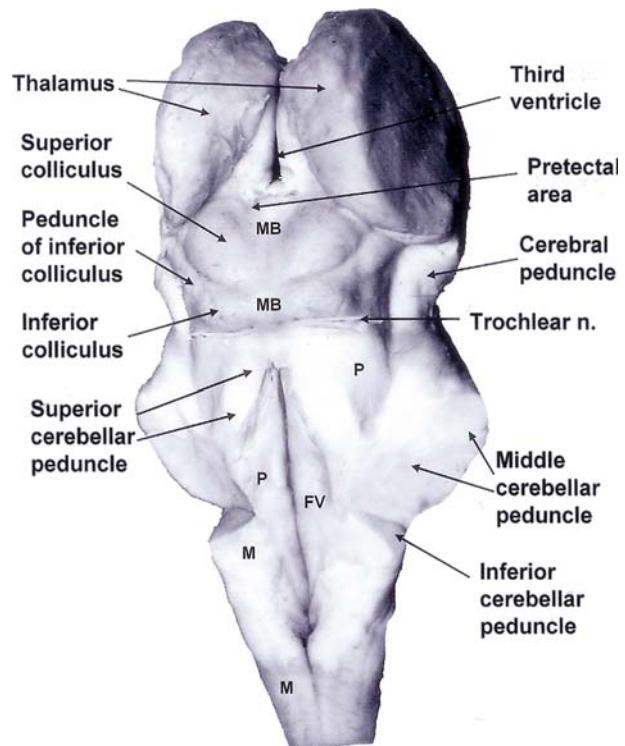
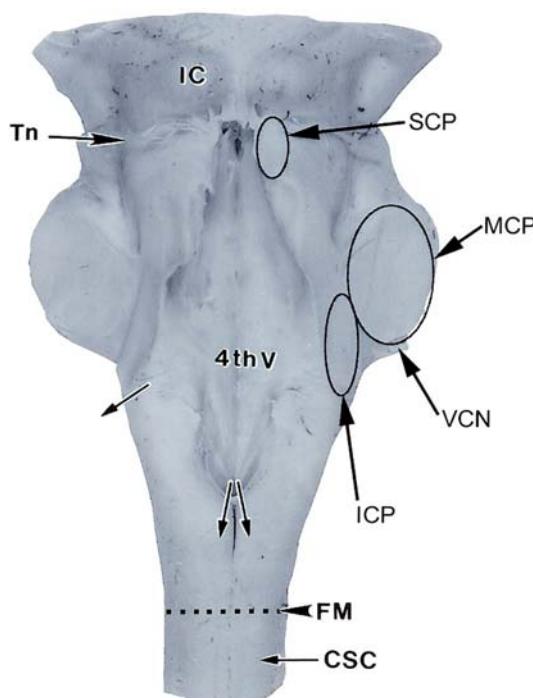


Fig. 2. (A, above) Posterior view of the brain stem with the cerebellum removed. The dotted line indicates the level of the foramen magnum (FM). The ovals mark the locations of the sectioned cerebellar peduncles: SCP, superior cerebellar peduncle; MCP, middle cerebellar peduncle; ICP, inferior cerebellar peduncle. The trochlear nerve (Tn) arises from the dorsal aspect of the brain stem. 4th V, fourth ventricle; CSC, cervical spinal cord; IC, inferior colliculus; VCN,

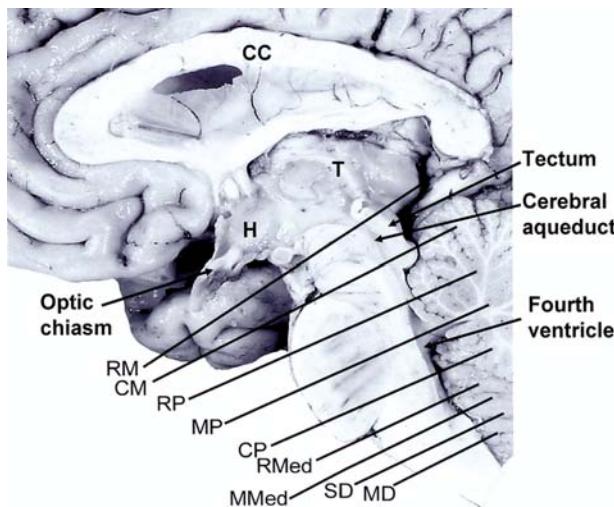


Fig. 3. Midsagittal section of the brain illustrating the diencephalon, brain stem, and cerebellum. The *labeled lines* correspond with the planes of the axial sections of the brain stem illustrated in Fig. 4, Fig. 5, Fig. 6, and Fig. 7. RM, rostral midbrain (Fig. 7B); CM, caudal midbrain (Fig. 7A); RP, rostral pons (Fig. 5C); MP, mid-pons (Fig. 5B); CP, caudal pons (Fig. 5A and Fig. 6); RMed, rostral medulla (Fig. 4D); MMed, mid-medulla (Fig. 4C); SD, sensory decussation of medulla (Fig. 4B); MD, motor decussation of medulla (Fig. 4A). CC, corpus callosum; H, hypothalamus; T, thalamus.

pia including a portion of the choroid plexus, extends superiorly from the medulla to the cerebellum and covers the V-shaped caudal aspect of the fourth ventricle. The *median aperture (of Magendie)* is located in the inferior medullary velum; through this opening cerebrospinal fluid passes from the *fourth ventricle* in to the subarachnoid space (Fig. 2A). The *cuneate and gracilis tubercles*, on the posterior aspect of the medulla, mark the locations of the *nucleus cuneatus* and *nucleus gracilis* (Fig. 4A, B). Here, terminals of the fasciculus gracilis and fasciculus cuneatus (dorsal columns of the spinal cord) synapse on neurons in the

Fig. 2. (A, above) (Continued)

vestibulocochlear nerve. The *small arrows* indicate the positions of the lateral (Luschka) and median (Magendie) apertures through which cerebrospinal fluid passes from the ventricular system into the subarachnoid space. **(B, below)** Posterior view of the brain stem and thalamus. The midbrain is continuous with the thalamus and hypothalamus (not shown) of the diencephalon. The cerebellum has been detached from the cerebellar peduncles. The third ventricle is continuous with the cerebral aqueduct of the midbrain and the fourth ventricle of the pons and medulla. The nuclei of the superior and inferior colliculi constitute the tectum of the midbrain. FV, fourth ventricle; M, medulla; MB, midbrain; P, pons. (Photo courtesy of Dr. Bruce Maley, University of Kentucky.)

nuclei gracilis and cuneatus. Their axons immediately cross the midline as the *sensory decussation* to form the contralateral *medial lemniscus* (Fig. 4B; see also Fig. 8B). This ascending sensory pathway carries discriminative tactile, vibratory, and proprioceptive sensations to the thalamus.

2.2. Pons

The anterior and lateral aspects of the base of the *pons* are formed by the fibers of the *middle cerebellar peduncles* (Fig. 1, Fig. 2, Fig. 5, and Fig. 6). These fibers originate from the *pontine nuclei* in the base of the pons, decussate forming the *pontocerebellar tract* and project to the contralateral cerebellum (Fig. 5 and Fig. 6). The pontine nuclei receive extensive inputs from motor areas of the cerebral cortex. The combined corticopontocerebellar pathway provides the cerebellum with information regarding planned and initiated motor movements originating in the motor cortex.

The slender *abducens nerves* emerge anteriorly from the pons-medulla boundary, course forward crossing the petrous ridge of the temporal bone into the middle cranial cavity, and subsequently enter the orbits to innervate the ipsilateral lateral rectus muscles (Fig. 1). The abducens nerves traverse the middle cranial cavity accompanied by the oculomotor, trochlear, and ophthalmic nerves; all are embedded in the lateral wall of the cavernous sinuses and pass through the superior orbital fissures into the orbits (see Fig. 1B in Chapter 13). In the intact brain, the basilar artery occupies the median depression on the anterior surface of the base of the pons (Fig. 1).

Three pairs of cranial nerves enter and exit the lateral aspect of the pons (Fig. 1). The *trigeminal nerves* emerge from the lateral aspect of the pons and course anteriorly crossing the petrous ridge of the temporal bones to the trigeminal ganglia in the middle cranial cavity. The three divisions of the trigeminal nerve, the ophthalmic, maxillary, and mandibular nerves, emerge from trigeminal ganglion and exit the skull through the superior orbital fissure, foramen rotundum, and foramen ovale, respectively (see Chapter 13 for additional illustrations).

The trigeminal nerve supplies sensory innervation to the ipsilateral face, oral and nasal cavities. The sensory nerves have their cell bodies in the trigeminal ganglion, and their central processes follow the trigeminal nerve to the pons. In addition, the trigeminal motor fibers follow the mandibular division supplying the muscles of mastication.

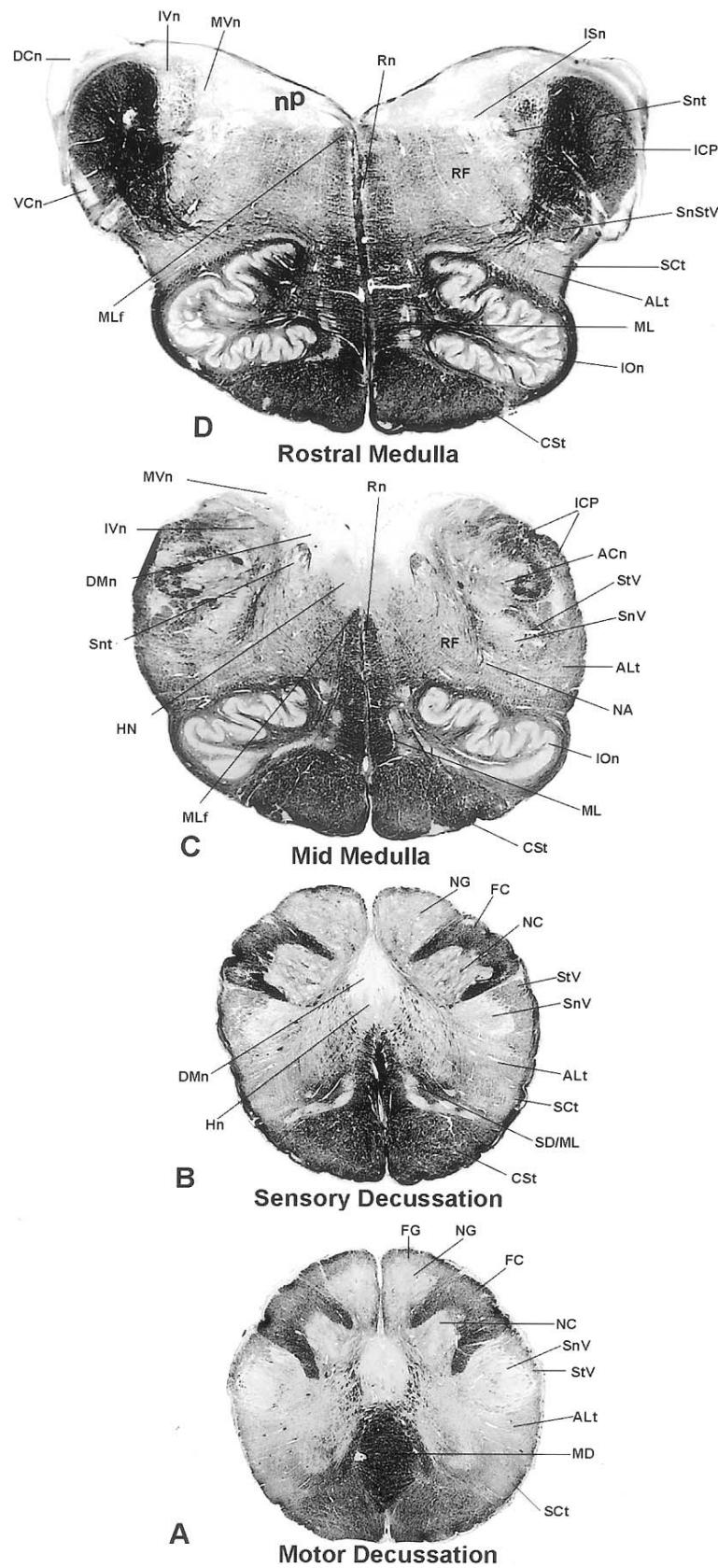


Fig. 4. (Continued)

The *facial and vestibulocochlear nerves* are attached to the lateral aspect of the pons-medulla junction (Fig. 1 and Fig. 5A). These nerves are in close relation to one another as they pass laterally in the posterior cranial cavity to enter the internal auditory meatus. In their lateral course, the facial and vestibulocochlear nerves cross the *cerebellopontine space or angle*, which is formed by the brain stem and the overlying cerebellum at the pons-medulla boundary. These nerves are also in close relation to the *flocculus* of the cerebellum, the *inferior cerebellar peduncle*, and the *lateral aperture of the fourth ventricle* (Fig. 1 and Fig. 2). The lateral apertures (of Luschka) and the median aperture (of Magendie), described later, are the only portals of exit for cerebrospinal fluid from the ventricular system into the subarachnoid space (see Chapter 8 for details).

The facial nerve conveys motor innervation to the ipsilateral facial muscles, taste from the anterior two-thirds of the tongue, and parasympathetic innervation to the lacrimal gland in the orbit and the salivary glands in the floor of the mouth. The facial nerve enters the skull through the stylomastoid foramen, follows the facial canal in the temporal bone, and enters the posterior cranial cavity through the internal auditory meatus on course to the brain stem. The vestibulocochlear nerve originates in the labyrinth and accompanies the facial nerve to the brain stem (Fig. 1, Fig. 2, Fig. 4D, and Fig. 5A).

2.3. Midbrain

The posterior aspect of the *midbrain* is characterized by two pairs of swellings, the *superior and inferior colliculi*, which are components of the visual and auditory systems, respectively (Fig. 2 and Fig. 3). The *pineal gland*, a component of the epithalamus of the diencephalon, rests in the midline between the superior colliculi. Just superior (rostral) to the superior colliculi is the *pretectal area*, which is functionally related to light reflexes (Fig. 2B). The *trochlear nerves* emerge from the brain stem immediately inferior (caudal) to

the inferior colliculi and innervate the superior oblique muscles in the orbit (Fig. 1 and Fig. 2). Inferior to the emerging trochlear nerves, the *superior cerebellar peduncles* enter the midbrain. These fibers are the principal output pathway from the cerebellum to the motor centers in the midbrain and diencephalon (Fig. 2).

The anterior surface of the midbrain is composed largely of the paired *cerebral peduncles*. These massive bundles of fibers, which include the corticonuclear and corticospinal tracts, connect cerebral cortical motor areas associated with motor functions to the motor neurons of the brain stem and spinal cord (Fig. 7B and Fig. 8B). The base of the midbrain between the cerebral peduncles is the *posterior perforated substance*, so named because small vessels penetrate here to supply intrinsic midbrain structures. In the intact brain, the arachnoid stretches between the cerebral peduncles forming the *interpeduncular cistern* (Fig. 1 and Fig. 7B).

The *oculomotor nerves* exit the base of the midbrain just medial to the cerebral peduncles (Fig. 1 and Fig. 7B). They pass anteriorly into the middle cranial cavity and enter the orbits through the superior orbital fissures along with the trochlear, abducens, and ophthalmic nerves. The oculomotor nerve carries motor innervation to ipsilateral extraocular muscles and parasympathetic innervation to the ipsilateral iris and ciliary muscle.

3. VENTRICULAR SYSTEM OF THE BRAIN STEM

During the embryonic period, the lumen of the neural tube develops into the ventricular system of the brain consisting of the paired lateral ventricles and unpaired third ventricle, cerebral aqueduct, and fourth ventricle. The *third ventricle*, located between the bilateral components of the *diencephalon*, is continuous caudally in the brain stem with the *cerebral aqueduct* and the *fourth ventricle* (Fig. 7). The cerebral aqueduct traverses the midbrain to the rostral border

Fig. 4. (Continued)

Axial sections of the human medulla corresponding with the planes illustrated in Fig. 3; silver stain, nerve fibers are darkly stained, and relatively unstained areas consist mostly of neuron cell bodies. Fourth ventricle is located posterior to (C) and (D). (A) Motor (pyramidal) decussation; (B) sensory (dorsal column) decussation; (C) mid-medulla; (D) rostral medulla. ACn, accessory cuneate nucleus; ALt, anterolateral tracts; CSt, corticospinal tract; DCn, dorsal (posterior) cochlear nucleus; DMn, dorsal motor nucleus; Fc, fasciculus cuneatus; Fg, fasciculus gracilis; Hn, hypoglossal nucleus; ICP, inferior cerebellar peduncle; IOn, inferior olfactory nucleus; ISn, inferior salivatory nucleus; IVn, inferior vestibular nucleus; MD, motor (pyramidal) decussation; ML, medial lemniscus; MLf, medial longitudinal fasciculus; MVn, medial vestibular nucleus; NA, nucleus ambiguus; NC, nucleus cuneatus; NG, nucleus gracilis; nP, nucleus prepositus; RF, reticular formation; Rn, raphe nuclei of reticular formation; Sct, spinocerebellar tracts; SD/ML, sensory decussation forming the medial lemniscus; Snt, solitary nucleus and tract; SnV, spinal trigeminal nucleus; StV, spinal trigeminal tract; VCn, ventral (anterior) cochlear nucleus.

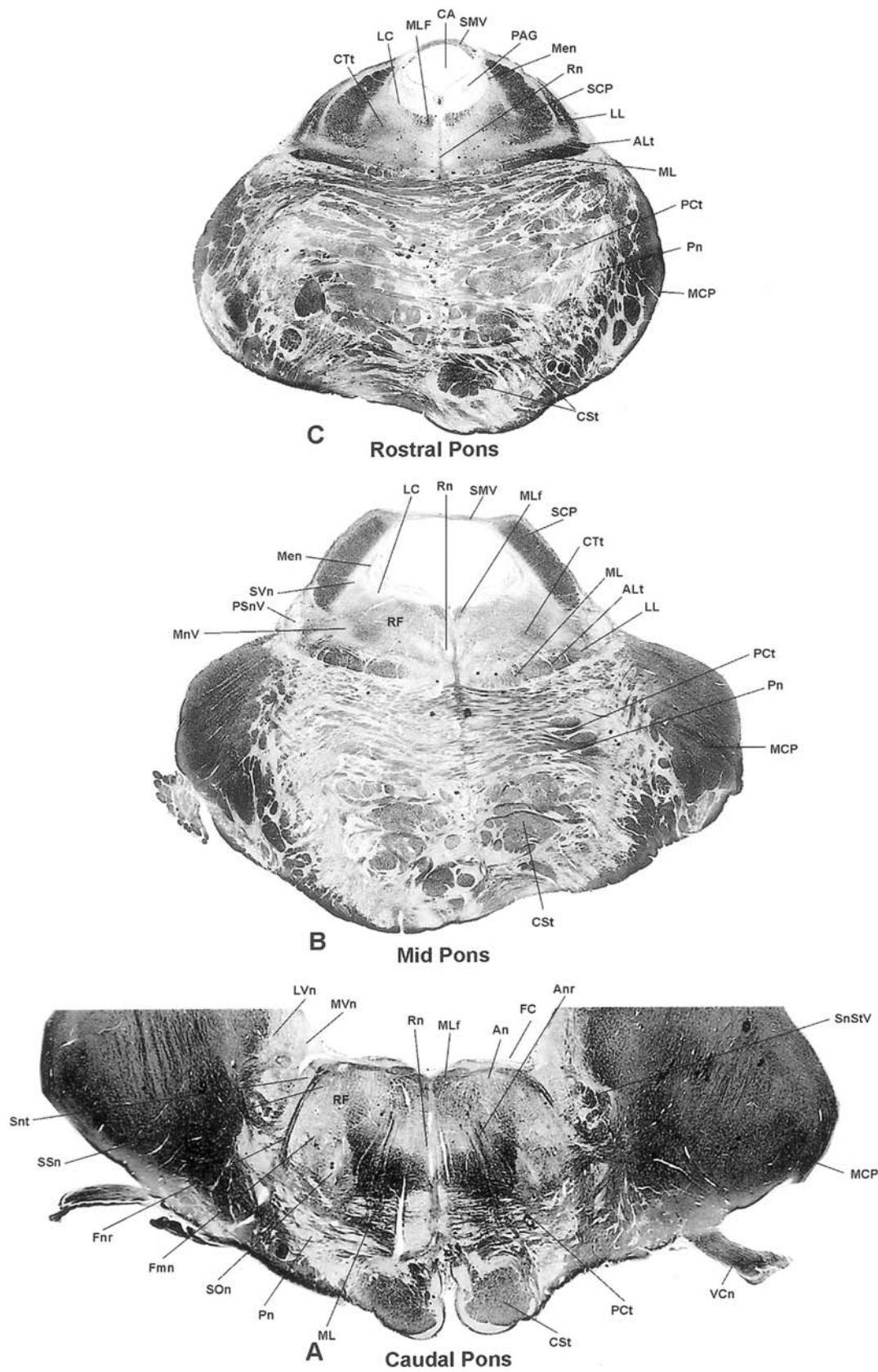


Fig. 5. (Continued)

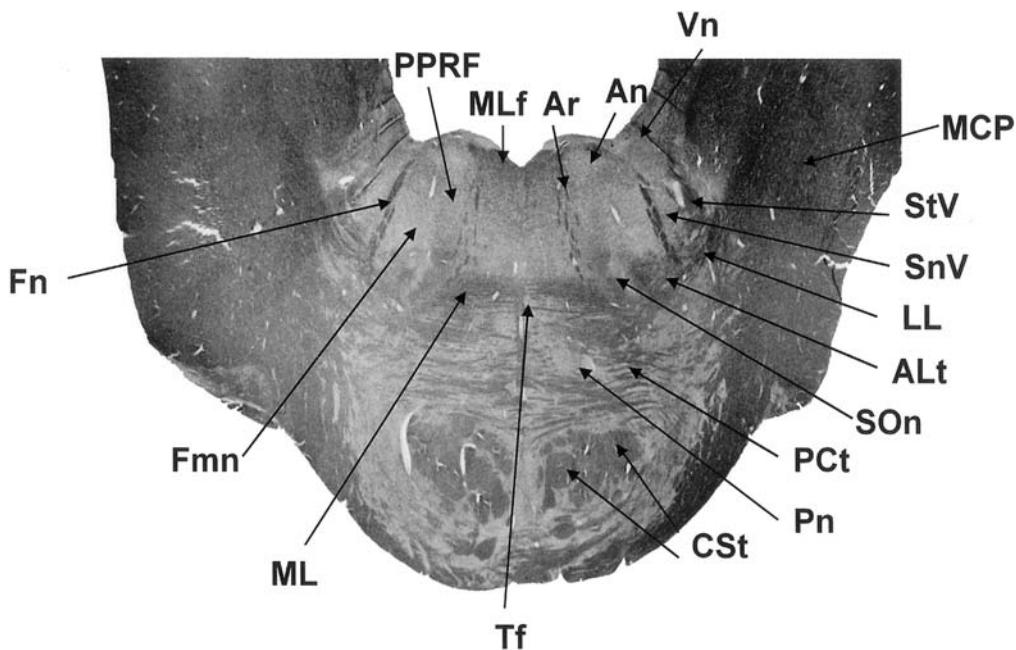


Fig. 6. Axial section of human pons approximately 1 cm rostral to Fig. 5A; luxol fast blue-neutral red stain, nerve fibers are darkly stained, and relatively unstained areas consist mostly of neuron cell bodies. ALt, anterolateral tracts; An, abducens nucleus; Ar, abducens nerve root; CSt, corticospinal tract; Fmn, facial motor nucleus; Fn, facial nerve root; LL, lateral lemniscus; MCP, middle cerebellar peduncle; ML, medial lemniscus; MLf, medial longitudinal fasciculus; Pct, pontocerebellar tract; Pn, pontine nuclei; PPRF, paramedian pontine reticular formation (pontine lateral gaze center); SnV, spinal trigeminal nucleus; SOm, superior olive nucleus; StV, spinal trigeminal tract; Tf, trapezoid fibers; Vn, vestibular nuclei. The facial colliculus is formed by the facial nerve fibers (Fn) arching over the abducens nucleus (An).

of the pons where it expands as the pyramidal-shaped fourth ventricle, which is located posterior to the pons and medulla (Fig. 2, Fig. 3, and Fig. 5B, C). Removal of the cerebellum reveals the floor of the fourth ventricle (rhomboid fossa), which extends into the base of the cerebellum posteriorly and is bounded by the anterior and posterior medullary veli of the cerebellum. The *anterior medullary velum* is continuous superiorly with the tectum of the midbrain. The *posterior medullary velum* is a thin membrane consisting of ependyma and choroid plexus and extends inferiorly to attach to the medulla (Fig. 3). The caudal extent of the fourth ventricle opens into the

subarachnoid space through the *median aperture (of Magendie)* in the posterior medullary velum; this aperture permits the passage of cerebrospinal fluid from the fourth ventricle into the *cerebellomedullary cistern (cisterna magna)*. At the pons-medullary boundary, the fourth ventricle extends laterally forming *lateral apertures (of Luschka)*; these apertures permit the passage of cerebrospinal fluid into the *pontine cistern* (Fig. 2A). Each lateral aperture opens into the pontine cistern in close association with the *flocculus* of the cerebellum and the roots of the *facial and vestibulocochlear nerves*. The median and lateral apertures are the only passages for

◀ **Fig. 5. (Continued)**

Axial sections of the human pons corresponding with the planes illustrated in Fig. 3; silver stain, nerve fibers are darkly stained, and relatively unstained areas consist mostly of neuron cell bodies. The fourth ventricle is located posterior to (A), the ventricle narrows (B) becoming continuous with the cerebral aqueduct (C). (A) Caudal pons, just rostral to the medulla-pons boundary; (B) mid-pons; (C) rostral pons. ALt, anterolateral tracts; An, abducens nucleus; Anr, abducens nerve root; CA, cerebral aqueduct; CSt, corticospinal tract; CTt, central tegmental tract; FC, facial colliculus; Fmn, facial motor nucleus; Fn, facial nerve root; LC, locus ceruleus; LL, lateral lemniscus; LVn, lateral vestibular nucleus; MCP, middle cerebellar peduncle; Men, mesencephalic nucleus of trigeminal; ML, medial lemniscus; MLf, medial longitudinal fasciculus; MnV, motor nucleus of trigeminal; MVn, medial vestibular nucleus; PAG, periaqueductal gray; Pct, pontocerebellar tract; Pn, pontine nuclei; PSnV, principal sensory nucleus of trigeminal; RF, reticular formation; Rn, raphe nuclei; SCP, superior cerebellar peduncle; SMV, superior medullary velum; Sn, spinal trigeminal nucleus; Snt, solitary nucleus and tract; SOm, superior olive nucleus; SSn, superior salivatory nucleus; StV, spinal trigeminal tract; VCn, vestibulocochlear nerve.

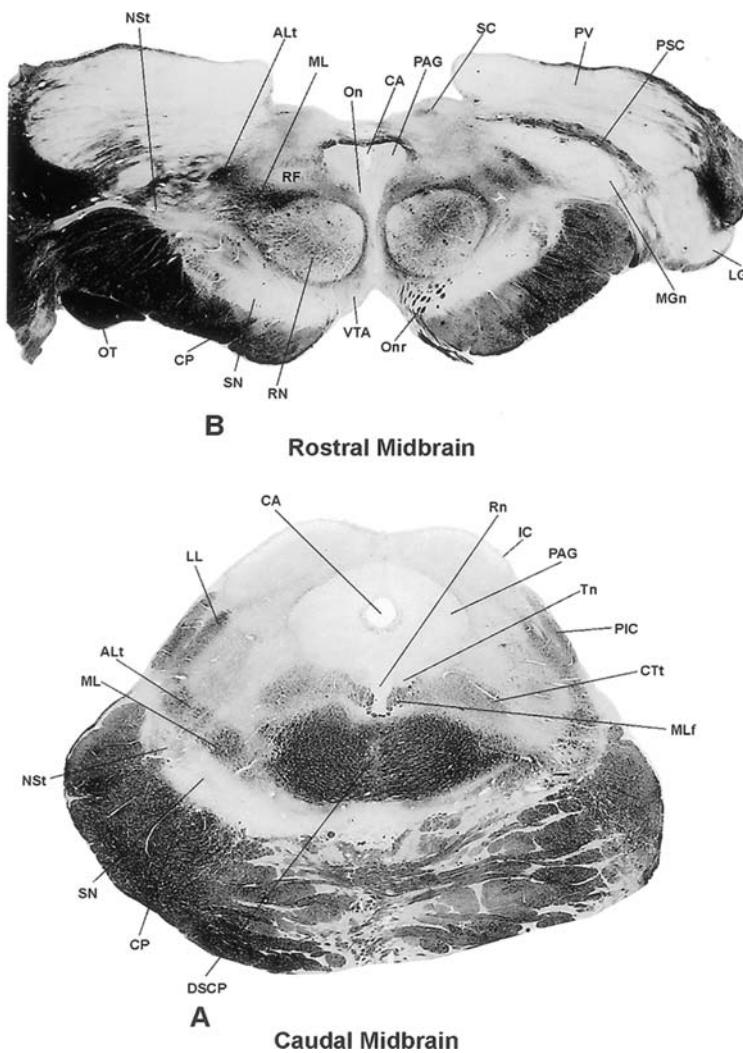


Fig. 7. Axial sections of the human midbrain corresponding with the planes illustrated on Fig. 3; silver stain, nerve fibers are darkly stained, and relatively unstained areas consist mostly of neuron cell bodies. V-shaped space between the cerebral peduncles (CP, Fig. 5B) corresponds with the interpeduncular space (cistern). **(A)** Caudal midbrain, level of the inferior colliculus (IC). **(B)** Rostral midbrain, level of the superior colliculus (SC). ALT, anterolateral tracts; CA, cerebral aqueduct; CP, cerebral peduncle; CTt, central tegmental tract; DSCP, decussation of the superior cerebellar peduncle; IC, inferior colliculus; LGN, lateral geniculate nucleus; LL, lateral lemniscus of the thalamus; MGN, medial geniculate nucleus of the thalamus; ML, medial lemniscus; MLf, medial longitudinal fasciculus; NSt, nigrostriate tract; On, oculomotor nucleus; Onr, oculomotor nerve root; OT, optic tract; PAG, periaqueductal gray; PIC, peduncle of the inferior colliculus; PCS, peduncle of the superior colliculus; PV, pulvinar of the thalamus; RF, reticular formation; Nn, red nucleus; RN, raphe nuclei of the reticular formation; Sc, superior colliculus; SN, substantia nigra; VTA, ventral tegmental area.

cerebrospinal fluid from the ventricular system into the subarachnoid space.

4. INTERNAL STRUCTURE OF THE BRAIN STEM

Sections of the brain stem reveal the anatomic relationships between the cranial nerve nuclei and nerves, reticular formation, and ascending and descending (long) tracts associated with motor and sensory

functions (Fig. 4, Fig. 5, Fig. 6, Fig. 7, and Fig. 8). Figure 3 locates the rostral-caudal plane of these axial sections. The *tectum* (L. roof) is that part of the midbrain posterior to the cerebral aqueduct and includes the superior and inferior colliculi. The *tegmentum* (L. covering structure) includes that part of the midbrain anterior to the cerebral aqueduct, except the cerebral peduncles (Fig. 2, Fig. 3, and Fig. 7). The pons and medulla do not have components of the tectum; the tegmentum of the pons and medulla includes all areas

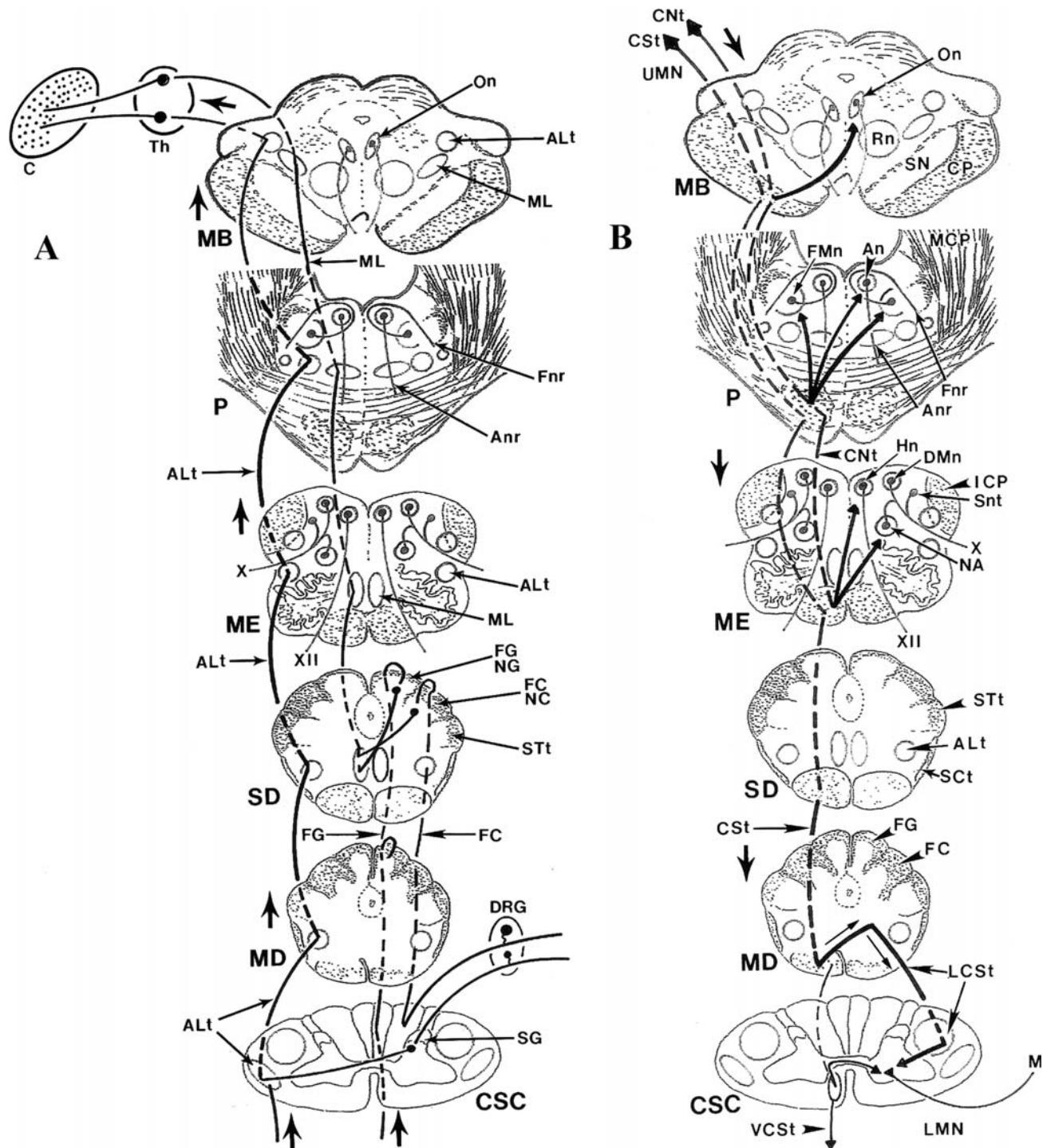


Fig. 8. Representations of the principal long (**A**) ascending and (**B**) descending tracts traversing the brain stem. CSC, cervical spinal cord; MB, midbrain; MD, motor decussation of the caudal medulla; ME, mid-medulla; P, pons; SD, sensory decussation of the caudal medulla. The courses of the long tracts are anatomically closely related to cranial nerve nuclei and nerve roots. (See Fig. 3 and Fig. 6 in Chapter 13 for illustrations of the trigeminal sensory and motor pathways in the brain stem.) ALT, anterolateral tracts; An, abducens nucleus; Anr, abducens nerve root; C, cerebral cortex; CP, cerebral peduncle; CNT, corticonuclear tract; CSt, corticospinal tract; DMn, dorsal motor nucleus of the vagus; DRG, dorsal root ganglion; FC, fasciculus cuneatus; FG, fasciculus gracilis; Fmn, facial motor nucleus; FnR, facial nerve root; Hn, hypoglossal nucleus; ICP, inferior cerebellar peduncle; LCSt, lateral corticospinal tract; LMN, lower motor neurons; M, skeletal muscles; MCP, middle cerebellar peduncle; ML, medial lemniscus; NA, nucleus ambiguus; NC, nucleus cuneatus; NG, nucleus gracilis; On, oculomotor nucleus; Rn, red nucleus; SCt, spinocerebellar tract; SG, substantia gelatinosa; SN, substantia nigra; Snt, solitary

anterior to the fourth ventricle except for the base of the pons and the pyramids (Fig. 5 and Fig. 6). Tectum and tegmentum are terms often used to designate locations of brain-stem tracts and nuclei.

Ascending sensory pathways from the spinal cord and the motor pathways descending from the cerebral cortex traverse the brain stem; these are referred to as *long tracts* (Fig. 8). The motor pathways activate musculature of the contralateral face, extremities, and trunk; the ascending pathways convey sensations from the contralateral side of the body. Along their courses through the brain stem, these long-tract axons come into close anatomic relation to cranial nerve nuclei and the emerging cranial nerves. The cranial nerves predominately innervate ipsilateral structures in the head and neck. Lesions often affect regions of the brain stem where long tracts and cranial nerve components are in close proximity. For example, cerebrovascular accidents (stroke) result from diminished blood flow in a branch of the vertebrobasilar system supplying a region of the brain stem. Consequently, neurologic deficits can be observed reflecting dysfunctions of one or more

Fig. 8. (Continued)

nucleus and tract; STt, spinal trigeminal tract; Th, thalamus; UMN, upper motor neurons; VSCT, ventral corticospinal tract; X, vagus nerve; XII, hypoglossal nerve. (A) Primary sensory neurons (DRG) convey pain, thermal, and general tactile modalities to the dorsal horn of the spinal cord (SG); axons of relay neurons cross to join the contralateral anterolateral tracts (ALT). The axons of other primary sensory neurons (DRG) convey fine tactile and conscious proprioception modalities via the ipsilateral dorsal columns (FC, FG) and terminate on the ipsilateral nuclei gracilis and cuneatus (NG, NC). Axons of NG and NC cross the midline at the sensory decussation in the caudal medulla (SD) and form the contralateral medial lemniscus (ML). ALT and ML terminate in the contralateral thalamus (Th), and thalamocortical fibers convey the sensory modalities to the postcentral gyrus. (B) Corticospinal (CSt) and corticonuclear (CNt) tracts originate from cortical motor neurons and descend the brain stem in a ventral position. The corticonuclear (CNt) upper motor neuron fibers terminate mostly on contralateral cranial nerve motor nuclei (MB, ME, P); some cranial nerve motor nuclei (Fmn) receive bilateral corticonuclear innervation (P). Corticospinal tract (CSt) crosses the midline at the motor decussation (MD) in the caudal medulla and continues descending the contralateral spinal cord as the lateral corticospinal tract (LCSt). LCSt terminations provide upper motor neuron innervation to the (LMN). A small portion of CSt continues uncrossed as the ventral corticospinal tract (VCSt). VCSt axons subsequently cross the midline in the spinal cord at their levels of termination on motor neurons that innervate posterior trunk musculature.

cranial nerves ipsilateral to the side of the vascular lesion, and sensory and motor symptoms observable on the contralateral side of the body reflect dysfunctions of one or more of the long tracts.

The *reticular formation* consists of several subgroups of nuclei, diverse in morphology, connectivity, and neurochemistry, located throughout the tegmentum of the brain stem (Fig. 4, Fig. 5, Fig. 6, and Fig. 7) (see Chapter 11 for details). Reticular formation nuclei are often named for their anatomic location, cell morphology, or function (e.g., pontine reticulotegmental nucleus, nucleus gigantocellularis, and lateral gaze center, respectively). Axons of the reticular formation form tracts (e.g., reticulothalamic tract, reticulospinal tract, central tegmental tract) that project their influences to the cerebral cortex, limbic structures, hypothalamus, cerebellum, brain stem, and spinal cord somatic motor and visceral nuclei. Reticular formation nuclei receive input from the periphery via collaterals from sensory pathways and from many regions of the cerebral cortex, limbic structures, and the cerebellum. Together, reticular formation circuits participate in regulation of the sleep cycle, consciousness, emotion and behavior, motor and visceral functions, and processing of sensory information including modulation of pain.

4.1. Descending Long Tracts in the Brain Stem

4.1.1. VOLUNTARY MOTOR PATHWAY

The *voluntary motor pathway* (pyramidal tract) arises from the motor areas of the cortex (Fig. 8B). Axons of these cortical neurons traverse the cerebral white matter, the posterior limb and genu of the internal capsule, and the midbrain where they occupy a central position in the *cerebral peduncle* (Fig. 7). These *corticounuclear* (formerly termed corticobulbar) and *corticospinal tracts* are *upper motor neurons* and innervate cranial nerve and the spinal cord *lower motor neurons*, respectively. Axons of the lower motor neurons follow cranial and spinal nerves and the peripheral nerves to innervate skeletal muscle. The corticonuclear and corticospinal tracts and their associated lower motor neurons constitute the descending *motor pathway*.

4.1.2. UPPER MOTOR INNERVATION TO THE SPINAL CORD

The motor pathway to the spinal cord maintains a ventral position as it traverses the brain stem. In the pons, motor pathway fibers are separated into groups of fascicles by the intersecting *pontine nuclei* and *pontocerebellar fibers* (Fig. 5, Fig. 6, and Fig. 8B). In the

medulla, the corticospinal tract motor fibers collect in an anteromedial position as the *pyramids* and continue their caudal course (Fig. 1 and Fig. 4). About 90% of the fibers cross to the contralateral side at the medulla-spinal cord boundary forming the *motor (pyramidal) decussation* (Fig. 4A and Fig. 8B). The decussating motor fibers loop posterolateral and continue into the lateral funiculus of the spinal cord as the *lateral corticospinal tract*. Some lateral corticospinal fibers terminate directly on ventral horn lower motor neurons others synapse on intermediate neurons in the ventral horn. Fibers of the lower motor neurons follow spinal and peripheral nerves and innervate the skeletal muscles of the extremities and trunk.

The 10% of motor pathway fibers that do not cross at the motor decussation form the *anterior (ventral) corticospinal tract* in the ventral funiculus of the spinal cord (Fig. 8B). These fibers decussate in the spinal cord at the level where they synapse on lower motor neurons. These motor neurons innervate axial musculature.

It is important to note that rostral to the motor decussation, the voluntary motor fibers traversing the brain stem serve the *contralateral side of the body*. Destruction of the voluntary motor pathway in the brain stem results in *contralateral hemiplegia*, whereas destruction of the lateral corticospinal tract in the spinal cord results in *ipsilateral hemiplegia* of musculature innervated below the level of the spinal cord lesion.

4.1.3. UPPER MOTOR INNERVATION TO CRANIAL NERVE MOTOR NUCLEI

Most fibers of the *corticounuclear tract* follow the corticospinal fibers as they traverse the brain stem. Although there is a bilateral corticounuclear innervation to most cranial nerve motor nuclei that innervate skeletal muscles in the head and neck, there is a predominance of crossed innervation (Fig. 8B). Consequently, destruction of one corticounuclear tract results in weakness (paresis) observed on the contralateral side of the face.

There are some exceptions to the bilateral innervation of certain cranial nerve motor nuclei. The most notable exception is the corticounuclear innervation of the *facial motor nucleus* (Fig. 6 and Fig. 8B). Here, only contralateral corticounuclear fibers terminate on those facial motor neurons that activate facial muscles of the lower half of the face. Facial motor neurons that innervate the upper half of the face (frontalis and orbicularis oculi muscles) receive bilateral corticounuclear innervation. As a result, destruction of one

corticounuclear tract, rostral to the facial motor nucleus, produces facial paralysis of only the contralateral lower half of the facial musculature. This observation is termed a *central facial palsy* and is observed contralateral to the site of the lesion. In contrast, a *lesion destroying the facial motor nucleus or facial nerve* would result in paralysis of all ipsilateral facial muscles and is termed a *peripheral facial palsy (paralysis)*. A peripheral facial palsy is often accompanied by loss of all ipsilateral facial nerve functions.

Oculomotor, trochlear, and abducens motor nuclei receive corticounuclear innervation predominately from the contralateral frontal and parietal eye fields. The innervation is indirect through intermediate neurons in the reticular formation and from the vertical and horizontal gaze centers in the midbrain and pons, respectively (Fig. 6 and Fig. 8B).

Corticounuclear innervation to the accessory nucleus in the cervical spinal cord is mostly uncrossed. Thus, a corticounuclear tract lesion results in loss of innervation to the ipsilateral sternocleidomastoid and trapezius muscles.

The *hypoglossal nucleus* and nerve innervate the extrinsic and intrinsic skeletal muscles of the ipsilateral tongue. Corticounuclear innervation is bilateral to the hypoglossal nuclei; however, those neurons innervating the genioglossus muscle receive predominately crossed innervation. The paired genioglossus muscles, together, protrude the tongue in the midline. Therefore, a corticounuclear lesion results in deviation of the tongue, when protruded, contralateral to the lesion. In contrast, a lesion of the hypoglossal nucleus or nerve results in deviation of the tongue ipsilateral to the lesion. Additionally, hypoglossal nuclear or nerve lesions result in atrophy of the tongue musculature, and often fasciculations are observed in the denervated tongue musculature. These are signs of a lower motor neuron lesion.

4.2. ASCENDING LONG TRACTS IN THE BRAIN STEM

4.2.1. SENSORY PATHWAYS

Pain, thermal, tactile, and proprioceptive sensations originating in integument, musculoskeletal structures, viscera, and blood vessels *follow primary afferent fibers* in peripheral nerves from the periphery to the spinal cord (Fig. 8A). Sensory fibers have their cell bodies in the *dorsal root ganglia* and their central processes terminate on *secondary neurons* in the dorsal horn of the spinal cord (see Chapter 9 for details). Axons of secondary neurons form *tracts* in the spinal cord and brain stem; these tracts convey the sensory

modalities to the thalamus for processing. Axons of tertiary neurons in the thalamus convey sensory information to the parietal cortex. Together, the neurons forming these three neuron chains are referred to as *pathways* (e.g., *pain and thermal pathway*, or *non-conscious proprioception pathway*) (Fig. 8A). It should be noted that the tertiary neurons in some pathways are located elsewhere in the central nervous system (e.g., in the cerebellum).

In like manner, primary sensory fibers in the trigeminal, facial, glossopharyngeal, and vagus nerves have their cell bodies in ganglia located along the course of each of these cranial nerves. Their central processes synapse on secondary neurons in brain stem nuclei associated with these cranial nerves.

4.2.2. PAIN, THERMAL, AND GENERAL TACTILE PATHWAYS

Pain (nociceptive), thermal, and general tactile pathway sensations are conveyed by peripheral nerves from all dermatome levels to the dorsal horn of the spinal cord where they synapse on secondary neurons as described above. The secondary neuron axons cross to the contralateral lateral funiculus of the cord and, without synapse, ascend the spinal cord forming the *anterolateral tract (ALT), or system (lateral and anterior spinothalamic tracts)* (Fig. 4, Fig. 5, Fig. 6, Fig. 7, and Fig. 8A). ALT traverses the brain stem and terminates on *tertiary neurons* in the ventral posterior lateral nucleus of the thalamus where sensory modalities are processed and integrated. The axons of the tertiary neurons, the *thalamocortical tract*, relay the sensory modalities to the parietal cortex for conscious appreciation of their character and localization on the body surface.

ALT traverses the medulla in a lateral position posterior to the inferior olfactory nucleus and near the medullary surface (Fig. 4 and Fig. 8A). In the pons, ALT is positioned in a more central position in the tegmentum and then moves to a posterolateral position in the midbrain tegmentum (Fig. 5, Fig. 6, Fig. 7, and Fig. 8). Through its course in the brain stem the ALT is conveying pain, temperature, and general tactile modalities from the *contralateral side of the body*.

4.2.3. NONCONSCIOUS (REFLEX) PROPRIOCEPTION PATHWAYS

Nonconscious proprioception is conveyed by primary sensory neurons from mechanoreceptors in the integument and musculoskeletal structures associated with the trunk and lower extremities to the dorsal horn of the spinal cord. Here secondary neuron axons form the

dorsal (posterior) and ventral (inferior) spinocerebellar tracts (DSCT and VSCT), which convey proprioceptive sensations to the cerebellum (see Chapter 10 for details).

DSCT axons ascend in a dorsolateral position just under the pia of the spinal cord. In the medulla, DSCT maintains a lateral position and enters the cerebellum via the *inferior cerebellar peduncle* (Fig. 2 and Fig. 4C, D).

Axons forming VSCT cross to the contralateral lateral funiculus of the cord, ascend through the brain stem, and enter the cerebellum via the *superior cerebellar peduncle*. In the cerebellar white matter, VSCT crosses back to the side from which it originated.

Primary sensory fibers carrying nonconscious proprioceptive sensations from the upper extremities and neck to the cerebellum enter the spinal cord and ascend the ipsilateral spinal cord, without synapse, in the fasciculus cuneatus. They synapse on secondary neurons in the *lateral (accessory) cuneate nucleus* in the medulla (Fig. 4C). Lateral cuneate axons form the *cuneocerebellar tract*, which conveys nonconscious proprioception from upper extremities and neck to the cerebellum via the *inferior cerebellar peduncle*.

4.2.4. DISCRIMINATIVE TACTILE, CONSCIOUS PROPRIOCEPTION PATHWAYS

Axons of pathways conveying *discriminative tactile, conscious proprioceptive (position) sense*, as well as *vibratory sense*, from mechanoreceptors in the periphery follow a similar course to the thalamus and to conscious levels in the parietal cortex. Central processes of the primary sensory neurons enter the ipsilateral *dorsal funiculus* at all spinal cord levels. The dorsal funiculus is subdivided into *fasciculus gracilis* and *fasciculus cuneatus* (Fig. 8A). Axons carrying sensations from the distal parts of the ipsilateral lower extremity are located most medial in the fasciculus gracilis. Axons from progressively more rostral dermatome levels accumulate in a medial to lateral arrangement such that those axons from rostral cervical dermatomes are located most lateral in the fasciculus cuneatus. By convention, fasciculus gracilis contains axons functionally related to the lower half of the ipsilateral body, and fasciculus cuneatus contains those associated with the upper half of the ipsilateral body. Fasciculus gracilis and cuneatus of both sides of the spinal cord, together, are referred to as the *dorsal columns*.

The somatotopic arrangement in the dorsal columns is retained as fasciculus gracilis and cuneatus fibers terminate in the caudal medulla on neurons of the *nucleus gracilis* and *cuneatus*, respectively

(Fig. 4A, B and Fig. 8A). The secondary axons of nucleus gracilis and cuneatus loop anteriorly in the tegmentum of the caudal medulla then cross to the contralateral side of the brain stem forming the *sensory decussation* (Fig. 4B and Fig. 8A). The decussating axons collect as the *medial lemniscus* (*ML*) and follow a rostral course conveying discriminative tactile and proprioceptive modalities from the *contralateral body* to the ventral posterior lateral nucleus of the thalamus for integration and relay to the parietal cortex (Fig. 4, Fig. 5, Fig. 6, Fig. 7, and Fig. 8A). In the medulla, the *ML* is located just posterior to the pyramids on either side of the midline. *ML* fibers conveying modalities from the contralateral foot are most anterior in the *ML*, and fibers related to the contralateral shoulder and neck are most posterior (Fig. 4 and Fig. 5).

As the *ML* enters the pons, it shifts position such that cuneate fibers are most medial and gracile fibers most lateral. This somatotopic organization of *ML* fibers is maintained through the midbrain to their terminals in the thalamus (Fig. 5 and Fig. 6).

It is important to note that the sensory tracts in the pons and midbrain are arranged in a medial to lateral orientation: medial lemniscus most medial, anterolateral tracts (ALT) are intermediate, and the lateral lemniscus is most lateral in position (Fig. 4, Fig. 5, Fig. 6, Fig. 7, and Fig. 8A). Medial lemniscus and the anterolateral tracts convey sensory modalities from the contralateral side of the body, and the lateral lemniscus carries auditory sensations from both cochleae.

Clinicians use the term *long tracts* as in *long tract signs* to refer to symptoms related to lesions affecting these tracts in the brain stem or spinal cord. The “long tracts” referred to are those conveying voluntary motor, pain, and thermal and proprioceptive modalities to and from the spinal cord. Thus, a lesion in the brain stem is likely to affect one or more of these long tracts resulting in observations of dysfunctions on the *contralateral side of the body*.

5. PERIPHERAL DISTRIBUTIONS OF THE CRANIAL NERVES

5.1. Olfactory Nerve

The *olfactory nerve* (*CN I*) is the most rostral of the cranial nerves and consists of several fascicles that are the axons of olfactory neurons located in the olfactory mucosa in the superior aspect of the nasal chamber. The olfactory nerve fascicles terminate in the olfactory bulb, which lies on the cribriform plate of the ethmoid bone. Axons of olfactory bulb neurons

are conveyed by the olfactory tract to the olfactory trigone on the inferior surface of the frontal lobe just anterior to the anterior perforated substance. The olfactory sensations are projected to the forebrain and medial-basal temporal lobe (see Section 6.1. for details).

5.2. Optic Nerve

The *optic nerve* (*CN II*) consists almost entirely of axons from the *ganglion neurons* in the retina. Ganglion neuron axons collect at the *optic disk* of the retina and pass through the sclera forming the optic nerve, which then passes through the optic canal at the posterior aspect of the orbit. Within the cranial cavity, the two optic nerves join in the midline forming the *optic chiasm* (Fig. 3). Those optic nerve axons originating from the medial (nasal) half of each retina cross in the optic chiasm to join axons from the lateral (temporal) half of the contralateral retina; together, they form the contralateral *optic tract*. Most optic tract axons terminate in the ipsilateral *lateral geniculate nucleus* of the dorsal thalamus; lateral geniculate neurons form the *geniculocalcarine tract* (*optic radiation*), which projects visual information to the medial aspect of the *occipital lobe*.

5.3. Oculomotor Nerve

The *oculomotor nerve* (*CN III*) emerges into the interpeduncular cistern from the base of the midbrain medial to the cerebral peduncles and lateral to the posterior perforated substance (Fig. 1 and Fig. 7B). The oculomotor nerve passes anteriorly between the posterior cerebral and superior cerebellar arteries and lateral to the posterior clinoid process in the middle cranial cavity. It is encased in the lateral wall of the cavernous sinus as it continues forward through the superior orbital fissure into the orbit (see Fig. 1B in Chapter 13). The oculomotor nerve provides motor innervation to the extraocular muscles, except the lateral rectus and superior oblique muscles. It also carries parasympathetic preganglionic fibers to the ciliary ganglion in the orbit; from here postganglionic parasympathetic fibers innervate the constrictor of the pupil and the ciliary muscle.

5.4. Trochlear Nerve

The *trochlear nerve* (*CN IV*) innervates only the superior oblique extraocular muscle (Fig. 1 and Fig. 2). It emerges from the brain stem immediately inferior (caudal) to the inferior colliculus of the midbrain and is the only cranial nerve to emerge from the posterior aspect of the brain stem. The trochlear

nerve then courses anteriorly around the brain stem. Near the posterior clinoid process, the trochlear nerve becomes encased in the lateral wall of the cavernous sinus; continuing anteriorly, it passes through the superior orbital fissure into the orbit.

5.5. Trigeminal Nerve

The *trigeminal nerve (CN V)* is the largest of the cranial nerves and supplies sensory innervation to the face, oral and nasal cavities, and motor innervation to muscles of *mastication*. The trigeminal nerve consists of a sensory root and a smaller motor root (Fig. 1). Together, the sensory and motor components exit the lateral aspect of the pons, coursing anteriorly and crossing the petrous ridge of the temporal bone to reach the *trigeminal ganglion* in the middle cranial cavity, which is the location of the primary sensory cell bodies (see Chapter 13 for details). The peripheral processes of the trigeminal ganglion neurons form the three divisions of the trigeminal nerve, the ophthalmic, maxillary, and mandibular nerves, which pass through the superior orbital fissure, foramen rotundum, and foramen ovale, respectively. The sensory trigeminal components innervate the face and structures in the oral and nasal cavities. The trigeminal motor fibers accompany the mandibular nerve as it exits the foramen ovale. These motor fibers innervate muscles of mastication and other muscles (see Section 6.5.4).

5.6. Abducens Nerve

The *abducens nerve (CN VI)* supplies only the *lateral rectus* extraocular muscle. It emerges from the anterior aspect of the brain stem at the border between the pons-medulla junction (Fig. 1, Fig. 5A, and Fig. 6). The abducens nerve crosses the petrous ridge, becomes encased in the lateral wall of the cavernous sinus, and passes anteriorly through the superior orbital fissure into the orbit.

5.7. Facial Nerve

The *facial nerve (CN VII)* emerges as two roots from the lateral aspect of the brain stem at the pons-medulla border anterior to the flocculus of the cerebellum (Fig. 1, Fig. 5A, and Fig. 6). The larger root provides innervation to muscles of facial expression, stapedius, and other muscles. The smaller root, the *nervus intermedius*, conveys parasympathetic preganglionic innervation to the lacrimal, sublingual, and submandibular salivary glands and sensory fibers carrying taste from the anterior two-thirds of the tongue. The two facial roots course laterally through

the internal acoustic meatus and canal to the geniculate ganglion where the cell bodies for the taste fibers are located. The motor fibers continue through the facial canal in the temporal bone and emerge from the *stylomastoid foramen* to distribute motor branches to facial muscles. The parasympathetic fibers follow the *great superficial petrosal nerve* or the *chorda tympani nerve* to the *pterygopalatine or submandibular parasympathetic ganglia*, respectively. Postganglionic fibers provide parasympathetic innervation to the lacrimal, submandibular, and sublingual glands.

5.8. Vestibulocochlear Nerve

The *vestibulocochlear nerve (CN VIII)* originates from the sensory neurons in the *spiral and vestibular ganglia* associated with the auditory and vestibular end organs. The central processes follow the internal acoustic canal, emerge from the internal acoustic meatus, and enter the lateral aspect of the brain stem posterior to the facial nerve and in close relationship to the flocculus of the cerebellum (Fig. 1, Fig. 2A, and Fig. 5A).

5.9. Glossopharyngeal Nerve

The *glossopharyngeal nerve (CN IX)* originates as several fascicles from the lateral aspect of the brain stem just inferior to the medulla-pons border and posterior to the olive (Fig. 1). The glossopharyngeal nerve provides sensory innervation to the mucosa of the middle ear cavity, oral pharynx, posterior one-third portion of the tongue (including taste), and the baroreceptors and chemoreceptors associated with the carotid artery. Parasympathetic preganglionic innervation is provided to the *parotid gland* through postganglionic relays in the *otic ganglion*.

5.10. Vagus Nerve

The *vagus nerve (CN X)* originates as 8 to 10 fascicles inferior to, and in line with, the origins of the glossopharyngeal nerve fascicles (Fig. 1). It provides sensory innervation to part of the skin of the external auditory canal, laryngeal pharynx, larynx, and the organ systems in the thorax and abdomen. The vagus nerve supplies parasympathetic innervation to the glands, mucosae, and smooth muscle of these same organ systems. It also supplies voluntary motor innervation to the striated muscles of the pharynx and larynx. It is important to note that the vagus nerve supplies lower motor neuron innervation to the striated *vocalis muscle* and other muscles associated with the articulation of speech.

5.11. Accessory Nerve

A few fascicles of the *accessory nerve (CN XI)* originate from the brain stem, in line with those of the vagus nerve; this component is referred to as the cranial portion of the accessory nerve. The cranial portion distributes with the vagus nerve, and most authors consider it to be part of the vagus. The much larger spinal portion of the accessory nerve originates from the upper five cervical spinal cord segments. The spinal portion courses rostrally along the lateral aspect of the cervical cord, through the foramen magnum, and joins the glossopharyngeal and vagus nerves (Fig. 1). Together with the glossopharyngeal and vagus nerves, the accessory nerve exits the cranial cavity through the jugular foramen. The accessory nerve supplies lower motor neuron innervation to the *trapezius* and *sternocleidomastoid* muscles in the neck.

5.12. Hypoglossal Nerve

The *hypoglossal nerve (CN XII)* arises from a line of rootlets emerging from the brain stem anterior to the olive (Fig. 1). It courses through the hypoglossal canal lateral to the foramen magnum and provides lower motor innervation to the intrinsic and extrinsic muscles of the tongue.

6. NUCLEAR COMPONENTS OF THE CRANIAL NERVES AND THEIR FUNCTIONS

6.1. Olfactory Nuclear Components

As described earlier (Section 5.1), the *olfactory pathway* begins with the olfactory neurons in the olfactory mucosa; these give rise to numerous fascicles of axons, the *olfactory fila*, which pass through the cribriform plate of the ethmoid bone to synapse in the *olfactory bulb*. These neurons are the homologues of the cranial nerves described in this section. Olfactory sensations are integrated in the olfactory bulb, and axons of the mitral and tufted neurons of the bulb form the “*olfactory nerve*,” which conveys processed olfactory information to the frontal and temporal lobes. Olfactory sensations are not directly processed in the thalamus as is the case for most other sensory modalities of the cranial nerves. Instead, the olfactory pathway projects first to the olfactory tubercle in the base of the frontal lobe and to the piriform and entorhinal areas of the parahippocampal gyrus. These cortical areas are the *primary olfactory cortex (POC)*; here, olfactory sensations are processed and reach consciousness. POC projections include those to hippocampus, thalamus

and hypothalamus, and amygdala. These projections integrate olfaction with behavior patterns and visceral functions associated with the brain stem and autonomic innervation (see Chapter 26 for details). Note that the structure usually referred to as “*olfactory nerve*” is actually a tract of the central nervous system and is not anatomically associated with the brain stem.

6.2. Optic Pathway and the Brain Stem

As described previously (Section 5.2), the *optic nerves* originate from the ganglion neurons of the retinas; they enter the cranial cavity through the optic canals and join to form the *optic chiasm* (Fig. 3). Fibers from the nasal (medial) quadrants of the retinas cross in the optic chiasm to join axons originating from the temporal (lateral) quadrants of the contralateral eye; together they form the *optic tracts* (see Chapter 22 for details). Most optic tract axons terminate in the lateral geniculate nucleus of the thalamus. Some axons terminate in the *superior colliculus* and the closely associated *prectal region* of the midbrain to serve visual and light reflex functions, respectively (Fig. 2B and Fig. 7). Another important projection conveys information regarding environmental light-dark cycles to the hypothalamus.

Note that the optic nerve is not attached to the brain stem. Further, the first neurons in the visual pathway are the rods and cones of the retina; they are the homologues of the cranial nerves described below. Therefore, the “*optic nerves*” are actually tracts of the central nervous system.

6.3. Oculomotor Nucleus

The *oculomotor nuclei* lie near the midline in the midbrain tegmentum just posterior to the medial longitudinal fasciculus and anterior to the cerebral aqueduct at the level of the superior colliculus (Fig. 7B). The oculomotor nerve arises predominantly from the ipsilateral *oculomotor nucleus* and innervates all ipsilateral extraocular muscles, except lateral rectus and superior oblique muscles, which are innervated by the abducens and trochlear nerves, respectively.

The oculomotor nucleus consists of subnuclei that innervate particular extraocular muscles. In addition, bilateral clusters of parasympathetic preganglionic neurons are located along the dorsal and superior aspects of the oculomotor nucleus. These neurons are the *visceral nucleus of the oculomotor* or *Edinger-Westphal nucleus*. Axons of these parasympathetic neurons accompany the oculomotor nerve to the orbit where they synapse on postganglionic neurons

in the ciliary ganglion. The ciliary ganglion provides parasympathetic postganglionic innervation to the ciliary muscle and the constrictor muscle of the iris.

Neurons of the oculomotor nucleus also innervate the *levator palpebrae superioris muscle* in the orbit, which elevates the upper eyelid. Collectively, the emerging axons of the oculomotor nucleus pass ventrally through the red nucleus and accumulate at the medial border of the cerebral peduncle.

Lesions of the oculomotor nucleus or nerve result in *ptosis*, *lateral strabismus*, and *mydriasis* in the ipsilateral eye due to loss of innervation to the levator palpebrae muscle, medial rectus muscle, and the constrictor of the iris, respectively. Introducing light into the affected eye results in a consensual (indirect) response of the contralateral pupil but no direct response in the affected eye. This confirms that the efferent limb of the light reflex pathway has been disrupted.

The oculomotor nucleus receives corticonuclear input from the *frontal and parietal eye fields* through connections in the nearby reticular formation and from the superior colliculus, medial longitudinal fasciculus, and pretectal area. Additional input is provided by the *rostral interstitial nucleus of the medial longitudinal fasciculus* and the *interstitial nucleus of Cajal*. These nuclei are located near the oculomotor nucleus along the border between the periaqueductal gray and the midbrain tegmentum; they serve as an integrating center for vertical gaze.

6.4. Trochlear Nucleus

The *trochlear nuclei* consist of small bilateral clusters of motor neurons embedded in the *medial longitudinal fasciculus* near the midline in the tegmentum of the midbrain at the level of the *inferior colliculus* (Fig. 7A). Trochlear axons course posterolaterally and inferiorly following the boundary of the *periaqueductal gray*. They decussate posterior to the *cerebral aqueduct* and emerge on the posterior aspect of the brain stem just inferior (caudal) to the inferior colliculi (Fig. 2). The trochlear nerves are the only cranial nerves that decussate. Consequently, the trochlear nuclei innervate the contralateral *superior oblique muscle*, but the trochlear nerve innervates the ipsilateral superior oblique.

6.5. Trigeminal Nuclei

The trigeminal nuclear complex is located in the lateral tegmentum of the brain stem and extends from the caudal midbrain through the pons, medulla, and

upper three cervical spinal cord segments. Sensory modalities from the face, oral and nasal cavities terminate on specific trigeminal subnuclei. The trigeminal motor component arises from the trigeminal motor nucleus located in the midpontine tegmentum. The trigeminal nerve, consisting of sensory fibers from all three trigeminal divisions and the motor fibers in the mandibular division, enters the lateral aspect of the pons and distributes to the trigeminal subnuclei as described in the following sections (Fig. 1; see Chapter 13 for details).

6.5.1. THE PRINCIPLE SENSORY NUCLEUS

The *principle (or chief) sensory nucleus (PSnV)* is located in the lateral tegmentum at the point where the trigeminal nerve enters the pons (Fig. 5B). Trigeminal fibers convey *discriminatory tactile, position sense (conscious proprioception), and vibratory sense* from the face, musculature innervated by the trigeminal nerve, and oral structures such as the temporomandibular joint and periodontal ligaments, and terminate in PSnV. Most secondary axons from PSnV cross at the level of the nucleus, join the contralateral *ventral trigeminothalamic tract*, and ascend to the *ventral posterior medial nucleus* of the thalamus. A smaller number of PSnV axons form the *dorsal trigeminothalamic tract*, which projects to the ipsilateral thalamus. Thalamocortical projections convey the discriminative tactile and position sense modalities to the *post central gyrus* and other sensory areas of the parietal cortex.

PSnV participates in various reflexes through its projections to the spinal trigeminal nucleus, trigeminal motor nucleus, and the reticular formation.

6.5.2. THE SPINAL TRIGEMINAL NUCLEUS

The *spinal trigeminal nucleus (SnV)* is located in the lateral tegmentum forming a continuous column of neurons extending from the mid-pons through the medulla and the first three cervical segments of the spinal cord where it overlaps the *substantia gelatinosa*. SnV receives prominent input from the central processes of the trigeminal ganglion neurons, which form the spinal tract of the trigeminal located just lateral to SnV (Fig. 4, Fig. 5A, and Fig. 6).

Three subnuclei of SnV are recognized, they are: *subnucleus oralis (SNo)*, *subnucleus interpolaris (SNI)*, and *subnucleus caudalis (SNC)* (Fig. 4 and Fig. 5A; see also Fig. 6 in Chapter 13). SNo is directly continuous, caudally, with the PSnV and extends to the border of the pons and medulla. SNo receives input from all sensory modalities and projects to the reticular formation, cerebellum, and thalamus; its connections

imply a role in trigeminal reflexes. The more caudal portion of SNo receives nociceptive, thermal, and general tactile input from the perioral area of the face, the teeth, and gingiva.

SNi is continuous with SNo and extends to the caudal medulla. SNi forms connections with other cranial nerve nuclei and the reticular formation for reflexes and also projects to the contralateral thalamus.

SNC extends from the obex in the caudal medulla into the first three cervical spinal cord segments where it overlaps the substantia gelatinosa. It receives nociceptive, thermal, and general tactile modalities from areas of the face posterior to the perioral region. Axons of SNC and SNi neurons decussate in the cervical spinal cord and medulla and form the ventral trigeminothalamic tract. The ventral trigeminothalamic tract is located near the medial lemniscus and ascends to the ventral posterior medial nucleus of the thalamus. Thalamocortical projections convey the nociceptive, thermal, and tactile modalities to the postcentral gyrus and other sensory areas of the cortex.

6.5.3. MESENCEPHALIC TRIGEMINAL NUCLEUS

The *mesencephalic trigeminal nucleus* (*Men*) consists of a thin column of primary sensory neuron cell bodies located along the border of the periaqueductal gray lateral to the fourth ventricle. The processes of these neurons form the *mesencephalic tract of the trigeminal*; together, the tract and nucleus extend superiorly to midbrain levels lying just medial to the *superior cerebellar peduncle* (Fig. 5B, C). The peripheral fibers (i.e., the mesencephalic tract) and Men convey nonconscious proprioceptive impulses from the musculoskeletal and oral structures innervated by the trigeminal nerve and project to the cerebellum, trigeminal motor nucleus, and brain stem reticular formation. Men connections imply functions associated with trigeminal and brain-stem reflexes.

6.5.4. MOTOR NUCLEUS OF THE TRIGEMINAL

The *motor nucleus of the trigeminal* (*MnV*) lies just medial to the principal sensory nucleus (Fig. 5B). The axons of these neurons follow the mandibular division of the trigeminal in its peripheral distribution and provide lower motor neuron innervation to the skeletal muscles innervated by the trigeminal nerve. The muscles innervated are the *muscles of mastication* (*temporalis, masseter, medial and lateral pterygoid muscles*), the *tensor tympani, mylohyoid, and anterior portion of the digastric muscles*. MnV neurons have reciprocal connections with the reticular formation and other

brain-stem nuclei; these connections support such reflexes as salivation, chewing, and swallowing.

Bilateral corticonuclear projections from cortical motor areas provide upper motor neuron and voluntary motor innervation to the MnV.

6.6. The Abducens Nucleus

The *abducens nucleus* is located in the tegmentum of the caudal pons. Abducens axons course ventrally through the pontine tegmentum and intersect fibers of the ascending *medial lemniscus* (Fig. 5A and Fig. 6). They incline somewhat inferiorly as they traverse the base of the pons intersecting the crossing *pontocerebellar fibers* and the descending *corticospinal (pyramidal) tracts*. The abducens nerve exits the brain stem ventrally at the pons-medulla boundary and courses forward to the orbit to innervate the ipsilateral *lateral rectus muscle* (Fig. 1).

A large subgroup of abducens nucleus neurons and the surrounding reticular formation provide axons for internuclear circuits. Most of these axons join the contralateral *medial longitudinal fasciculus* and project principally to those oculomotor neurons that innervate the contralateral medial rectus muscle (Fig. 5, Fig. 6, and Fig. 7). Collectively, the abducens neurons and the surrounding *paramedial pontine reticular formation* (PPRF) are the *lateral gaze center* because they regulate conjugate eye movements in the horizontal plane (Fig. 5A and Fig. 6). Projections primarily from the *vestibular nuclei* to the abducens nucleus and PPRF are an important link in the *vestibulo-ocular reflex*, which integrates head and conjugate eye movements. Abducens nucleus and PPRF also have connections with the nucleus prepositus (hypoglossi), which is located in the pons-medulla tegmentum rostral to the hypoglossal nucleus (Fig. 4D). This circuit functions to stabilize eye position in the horizontal plane. Finally, the abducens nucleus and the PPRF receive corticonuclear projections mostly from the frontal and parietal eye fields. These cortical projections participate in generating saccade, pursuit, and voluntary eye movements.

Lesions affecting the abducens nucleus or abducens nerve can also disrupt the vestibulo-ocular reflex and, in addition, the ipsilateral eye assumes a medial position (*medial strabismus*) because of paralysis of the ipsilateral lateral rectus muscle.

6.7. Facial Nerve Nuclei

6.7.1. FACIAL MOTOR NUCLEUS

The *facial motor nucleus* is located in the tegmentum of the caudal pons and consists of lower motor

neurons destined to innervate muscles of the ipsilateral face. The muscles innervated by the facial motor nucleus are the *muscles of facial expression (mimetic muscles)*, *stapedius*, *stylohyoid*, *orbicularis oculi*, *frontalis*, *posterior portion of the digastric*, and *platysma* muscles (Fig. 5A and Fig. 6). In the tegmentum, facial motor axons first follow a posterior medial course looping around the abducens nucleus then follow an anterior lateral course passing between the facial nucleus medially and the spinal trigeminal nucleus laterally to exit at the pons-medulla boundary.

Each facial motor nucleus receives bilateral upper motor neuron corticonuclear innervation. It is important to note that those facial motor neurons that innervate the frontalis and orbicularis oculi muscles receive bilateral corticonuclear projections, whereas those motor neurons innervating the muscles of the lower half of the face receive predominately crossed corticonuclear innervation. Lesions involving the corticonuclear tract rostral to the facial motor nucleus (e.g., a lesion of the cerebral peduncle, genu of the internal capsule, or the face area of the pre-central gyrus) result in a *central facial palsy*. Here, innervation to the *lower half of the contralateral face* is lost, but the bilateral corticonuclear innervation of the facial motor nuclei spares innervation to the frontalis and orbicularis oculi.

In contrast, lesions destroying the facial motor nucleus or facial nerve result in a *peripheral facial palsy*, which consists of a lower motor neuron paralysis of *all ipsilateral muscles* innervated by the facial nerve. In addition, lesions of the facial motor nucleus or facial nerve can result in loss of parasympathetic innervation to the lacrimal, submandibular, and sublingual glands and taste from the anterior two-thirds of the tongue. Patients may complain of *hyperacusis* (sounds are abnormally loud) because of loss of innervation to the *stapedius* muscle located in the middle ear cavity. Facial motor nucleus or facial nerve lesions will also interfere with the *corneal response*. Here, the ipsilateral eye will not blink when the cornea is touched, and the eye cannot be tightly closed upon command.

The facial motor nucleus has connections through the reticular formation with nuclei associated with the trigeminal, glossopharyngeal, vagus, and hypoglossal nerves; these circuits support a variety of brain-stem reflexes.

The facial nerve also conveys taste sensations from the anterior two-thirds of the ipsilateral tongue. The taste fibers have their cell bodies in the *geniculate ganglion* located in the temporal bone, and their

central processes enter the *solitary tract* and synapse on neurons of the *solitary nucleus* in the caudal pons (Fig. 5A). Axons of the solitary nucleus provide brain stem connections for visceral reflexes. Other solitary neurons project taste sensations to the posterior ventral medial nucleus of the thalamus.

6.7.2. THE SOLITARY NUCLEUS AND TRACT

The *solitary nucleus and tract (Snt)* is the principle visceral sensory nucleus in the medulla and pons (Fig. 4C, D and Fig. 5A). Central processes of visceral afferent fibers in the facial, glossopharyngeal, and vagus nerves contribute to the solitary tract and synapse on solitary neurons. There are functional localizations in the solitary nucleus, for example, the most rostral part is associated with gustatory processing whereas the more caudal portion participates in cardiovascular and respiratory regulation. Brain-stem projections of the solitary nucleus include to the reticular formation, brain-stem parasympathetic preganglionic neurons, hypothalamus, and thalamus; these connections provide circuits that participate in autonomic regulation.

6.7.3. SUPERIOR SALIVATORY NUCLEUS

Parasympathetic preganglionic fibers of the facial nerve originate from the small *superior salivatory nucleus* in the lateral tegmentum of the caudal pons (Fig. 5A). Their axons follow the branches of the facial nerve and terminate on pterygopalatine and submandibular ganglia neurons in the face and floor of the oral cavity, respectively (Section 5.7). The post-ganglionic parasympathetic fibers innervate the lacrimal gland, mucosa of the oral and nasal cavities, and the submandibular and sublingual salivary glands.

6.8. Vestibular and Cochlear Nuclei

Two distinct components form the *vestibulocochlear nerve: the cochlear nerve and the vestibular nerve* (Fig. 1, Fig. 2, and Fig. 5A). Both components are the central processes of sensory neurons located in the *vestibular ganglia* associated with the labyrinth and *spiral ganglia* of the cochlea. Both components enter the posterior cranial cavity to terminate in the vestibular or cochlear nuclei on the lateral aspect of the medulla near the pons-medulla boundary.

6.8.1. VESTIBULAR NUCLEI

The *vestibular nerve* terminals synapse on neurons in the vestibular nuclei, which occupy a considerable area in the lateral tegmentum of the medulla and pons. *Superior, lateral, medial, and inferior vestibular nuclei* are recognized based on their neuron morphology and

connections (Fig. 4C, D, Fig. 5A, B, and Fig. 6). The *semicircular canals* project predominately to the more rostral components of the vestibular nuclear complex whereas the *utricle* and *saccule* project more prominently to the caudal components. The utricle and saccule sense the orientation of the body with respect to gravity, and the semicircular canals are responsive to rotational movements and acceleration.

The vestibular nuclei project to the *flocculonodular lobe* of the cerebellum by way of the *inferior cerebellar peduncle* (Fig. 1). The vestibulo-cerebellar projections convey information to the cerebellum about the orientation and movement of the body in space for integration with proprioceptive and other cerebellar inputs.

Other projections of the *vestibular nuclei* course rostrally to the abducens nucleus, nucleus prepositus, and the paramedial pontine reticular formation (PPRF) (Fig. 6). These nuclei project, via the *medial longitudinal fasciculus* (MLF), to the abducens, trochlear, and oculomotor nuclei and participate in coordinating conjugate eye movements with respect to head and body positions (Fig. 4, Fig. 5, and Fig. 6).

Lesions (e.g., stroke, demyelinating disease such as *multiple sclerosis*) affecting the *medial longitudinal fasciculus* (MLF) disrupt the vestibulo-ocular reflex. As a result, conjugate eye movements with respect to head movements in the horizontal plane are affected. Accordingly, if MLF on the right is lesioned, the patient's gaze to the left will be abnormal because the right eye will not adduct. The patient will also experience diplopia on attempted gaze to the left. Gaze to the right is normal unless MLF on both sides is lesioned.

MLF also projects caudally into the ventral funiculus of the spinal cord as the *medial vestibulospinal tract*; this projection coordinates head and body posture with conjugate eye movements. A prominent caudal projection of axons from the lateral vestibular nucleus is the *lateral vestibulospinal tract*; these axons synapse on spinal cord motor neurons that innervate the *antigravity musculature* thereby facilitating an upright posture.

6.8.2. COCHLEAR NUCLEI

Cochlear nerve fibers terminate on neurons of the *dorsal and ventral cochlear nuclei* located on the lateral aspect of the *inferior cerebellar peduncle* (Fig. 4D). There is a tonotopic organization in the cochlear nuclei such that cochlear fibers conveying low frequency sounds terminate in the superficial regions of the cochlear nuclei and those conveying high-frequency sounds terminate in the deep regions of the cochlear nuclei, respectively. The cochlear nuclei contain several types of neurons, and important

processing of auditory input occurs before projection to higher centers.

There are both crossed and uncrossed rostral projections from the cochlear nuclei. The crossing axons form the *trapezoid fibers (body)*, which interdigitate with the ascending fibers of the *medial lemniscus and anterolateral tracts* (Fig. 5A). The ascending auditory fibers collect as the *lateral lemniscus* just lateral to the prominent *superior olfactory nucleus* in the caudal pons (Fig. 5A and Fig. 6). The superior olfactory nucleus is also tonotopically organized; it integrates auditory impulses from the two ears and therefore plays an important role in localizing sound in the environment. The *lateral lemniscus* conveys auditory sensations from both ears to the *inferior colliculus* for reflex connections. The auditory modality is relayed, via the peduncle of the inferior colliculus, to the *medial geniculate nucleus* of the thalamus (Fig. 2B, Fig. 5B, C, Fig. 6, and Fig. 7).

6.9. GLOSSOPHARYNGEAL NUCLEI

The *glossopharyngeal nuclei* are located in the rostral medulla and include *inferior salivatory nucleus*, *solitary nucleus*, and the *nucleus ambiguus* (Fig. 4C, D).

6.9.1. INFERIOR SALIVATORY NUCLEUS

Parasympathetic preganglionic fibers of the glossopharyngeal nerve originate from the indistinct *inferior salivatory nucleus* located near the floor of the *fourth ventricle* (Fig. 4D). They synapse on post-ganglionic parasympathetic neurons in the otic ganglion and provide secromotor innervation to the ipsilateral *parotid gland*.

6.9.2. NUCLEUS AMBIGUUS

The *nucleus ambiguus* in the tegmentum of the medulla gives origin to the small component of glossopharyngeal motor fibers that innervate the striated *stylopharyngeus muscle* (Fig. 4C).

6.9.3. SOLITARY NUCLEUS

Sensory neurons are located in two small ganglia associated with the proximal end of the glossopharyngeal nerve. These are the primary sensory neurons that convey taste and other sensations from the posterior third of the ipsilateral tongue, middle ear cavity, and the oral pharynx. The sensory fibers from the oral pharynx constitute the *afferent limb of the gag (palatal) reflex*.

A subgroup of glossopharyngeal sensory fibers, the carotid nerve, carries *baroreceptor and chemoreceptor impulses* from the ipsilateral carotid sinus and body. The baroreceptor and chemoreceptor

fibers have their primary sensory cell bodies in the inferior glossopharyngeal ganglion, and their central processes join the *solitary tract* finally terminating in the *solitary nucleus* (Fig. 4C, D and Fig. 5A). These glossopharyngeal afferent projections to the solitary nucleus, along with similar projections from the vagus nerve, play a key role in the regulation of vital cardiovascular and respiratory functions (see Chapter 11 for details).

Solitary neurons also project to the brain stem reticular formation and other cranial nerve nuclei to provide circuits for visceral reflexes such as swallowing and salivation. The more rostral portion of the solitary nucleus projects glossopharyngeal taste sensations to the thalamus, and thalamocortical fibers convey taste sensations to the postcentral gyrus.

6.10. Vagal Nuclei

The nuclei of the *vagus nerve* are located in the tegmentum of the mid-medulla and include *nucleus ambiguus*, *dorsal motor nucleus of the vagus*, and the *solitary nucleus* (Fig. 4C).

6.10.1. DORSAL MOTOR NUCLEUS

The *dorsal motor nucleus* is located ventral to the floor of the *fourth ventricle* and lateral to the *hypoglossal nucleus* (Fig. 4C). Axons from the parasympathetic preganglionic dorsal motor nucleus terminate on postganglionic neurons associated with the cardiovascular, respiratory, and the gastrointestinal systems. The postganglionic parasympathetic fibers innervate mucosae, glands, cardiac and smooth muscle.

6.10.2. NUCLEUS AMBIGUUS

The heart is also innervated by parasympathetic preganglionic neurons located in or near the *nucleus ambiguus* (Fig. 4C). In addition, motor neurons in *nucleus ambiguus* innervate striated muscles of the *larynx, pharynx, and palate* including the vocal muscles. Fibers from *nucleus ambiguus* provide the *efferent limb of the gag (palatal) reflex*.

6.10.3. SOLITARY NUCLEUS

The *vagus nerve* contains sensory fibers whose cell bodies are located in small ganglia associated with the proximal end of the nerve near the jugular foramen. The visceral sensory fibers have their sensory cell bodies in the inferior vagal ganglion and their central processes join the *solitary tract* finally terminating in the *solitary nucleus* (Fig. 4C). This vagal sensory projection conveys sensations from the organs innervated by the *vagus*, including the baroreceptors in

the aortic arch. Vagal sensory fibers in the solitary tract terminate on the *solitary nucleus*, which projects to the reticular formation and other cranial nerve nuclei providing circuits for visceral reflexes.

It should be noted that the facial, glossopharyngeal, and vagus nerves contain small components of sensory fibers that innervate skin associated with the auricle and external auditory canal. These fibers join the trigeminal system in the brain stem (see Chapter 13 for details).

Ipsilateral lesions of the glossopharyngeal or vagus nerves usually result in an ipsilateral diminished gag reflex, difficulty in swallowing, and dysarthria. These symptoms are part of the *lateral medullary (Wallenberg) syndrome*.

6.11. Accessory Nucleus

The *accessory nerve* motor neurons are located in the *accessory nucleus* in the ventral horn of the cervical spinal cord. Accessory nerve rootlets emerge from the lateral aspect of the cervical cord and coalesce as a well defined nerve as they ascend superiorly in the subarachnoid space (Fig. 1). The accessory nerve passes through the foramen magnum into the posterior cranial cavity where it joins the vagus and glossopharyngeal nerves. Together, the three nerves exit through the jugular foramen into the neck.

The accessory nerve provides voluntary lower motor neuron innervation to the ipsilateral *sternocleidomastoid and trapezius muscles*. As a result of this circuitous course, spinal accessory nerve function can be diminished or lost as a result of lesions affecting the cervical cord, structures in the posterior cranial cavity, or trauma to the lateral aspect of the neck. Symptoms include weakness in turning the head to the contralateral side and lifting the ipsilateral shoulder.

6.12. Hypoglossal Nucleus

The *hypoglossal nucleus* is a prominent group of motor neurons extending throughout the dorsal aspect of the caudal medulla between the midline and the dorsal motor nucleus of the *vagus* (Fig. 4B, C). Hypoglossal axons course ventrally just lateral to the *medial lemniscus* and emerge from the medulla between the *pyramids* and the *inferior olive* (Fig. 1). The rootlets combine as the *hypoglossal nerve*, which passes through the hypoglossal canal and innervates the ipsilateral intrinsic skeletal muscles of the tongue. Destruction of the hypoglossal nucleus, its root or the hypoglossal nerve results in lower motor neuron paralysis of the ipsilateral half of

the tongue. Hypoglossal lesions result in deviation of the tongue toward the side of the lesion, when protruded, because of the paralysis of the ipsilateral genioglossus muscle. In addition, atrophy and fasciculations of the affected tongue musculature are observed.

7. CEREBELLUM

The *cerebellum* lies posterior to the pons and medulla and is connected to the brain stem by the paired *superior, middle, and inferior cerebellar peduncles* (Fig. 1; see Chapter 10 for details). The cerebellum consists of *lateral hemispheres*, further divided into anterior and posterior lobes, a midline *vermis*, and the flocculonodular lobe on the inferior surface. Superiorly, the cerebellar hemispheres contact the undersurface of the *tentorium cerebelli*. Inferiorly, the cerebellum occupies the intracranial depressions formed by the occipital bone.

The inferior aspect of the cerebellar hemispheres reveals two paired subdivisions, the *tonsils* and the *flocculonodular lobes* (Fig. 1). The flocculonodular lobes are formed by the *nodulus*, the most inferior portion of the vermis, and its bilateral extensions the *flocculi* (singular, *flocculus*). The flocculonodular lobe serves an important role in vestibular function.

The *cerebellar tonsils* are inferiorly directed protuberances of the cerebellar hemispheres that overlie the lateral edge of the *foramen magnum*. In clinical situations resulting in increased pressure in the posterior cranial cavity (i.e., a hemorrhage or an expanding tumor), this portion of the cerebellum can wedge between the rim of the foramen magnum and the medulla-spinal cord junction. This situation is termed *herniation of the cerebellar tonsils*. Cerebellar tonsil herniation can be fatal because it interferes with blood flow to the vital cardiovascular and respiratory neural centers in the medulla and with descending reticular formation axons that regulate sympathetic innervation to cardiovascular and respiratory systems.

8. OTHER PROMINENT BRAIN STEM STRUCTURES

Other prominent structures in the brain stem are described in this section; they play important roles in regulation and integration of motor, limbic, and visceral functions. In addition, the reticular formation nuclei and tracts are distributed throughout the brain stem (see Section 4 and Chapter 11).

8.1. Substantia Nigra

Substantia nigra neurons are located just posterior to the cerebral peduncle (Fig. 7B). Two subgroups of neurons are recognized: a *reticular region (pars reticulata)*, located just deep (posterior) to the cerebral peduncle; and a *compact region (pars compacta)*, located deep (posterior) to the reticular region. Neurons of the compact region contain the dark pigment melanin, which is a by-product of the dopamine synthesis pathway. Neurons of the compact region are *dopaminergic* and have reciprocal connections with the *neostriatum* via the *nigrostriate and striatonigral tracts*. Dopamine can facilitate or inhibit neostriatal targets depending on the dopamine receptor type expressed by those targets. Degeneration of substantia nigra compact region neurons deprives the neostriatum of the influences of dopamine and results in *Parkinson's disease*.

Neurons of the reticular region use *GABA* as a transmitter, their principal projections are inhibitory to the neostriatum, thalamus, and reticular formation. Both groups of neurons are involved in regulation of motor activities.

8.2. Red Nucleus

The *red nucleus* is a prominent feature of the midbrain tegmentum at the level of the superior colliculus (Fig. 7B). The red nucleus receives ipsilateral, somatotopically organized projections from ipsilateral cortical motor areas (corticorubral tract) and a prominent input from the contralateral cerebellum via the *superior cerebellar peduncle* (Fig. 2 and Fig. 5B, C).

The superior cerebellar peduncles consist mostly of axons arising from the paired *dentate nuclei of the cerebellum* and are a principal output pathway of the cerebellum. They course along the posterolateral aspects of the rostral pons, then enter the pons forming the *decussation of the superior cerebellar peduncles* in the midbrain tegmentum at the level of the inferior colliculus (Fig. 7A). Some decussated fibers terminate in the contralateral red nucleus, whereas most fibers continue a rostral course to terminate in the contralateral thalamus. Collectively, these fibers are the *dentatorubrothalamic tract* and constitute a principal pathway by which the cerebellum and red nucleus participate in regulation of ongoing motor activities.

Red nucleus axons also project to the motor neurons in the brain stem and spinal cord as the contralateral *rubronuclear and rubrospinal tracts*. These inputs have facilitatory influences on motor neurons that innervate flexor muscle groups. In this regard, these pathways to brain stem and spinal cord oppose

the lateral vestibulospinal tract facilitatory influence on the extensor muscles, especially the antigravity muscles.

8.3. Inferior Olivary Nucleus

The *inferior olfactory nucleus* receives a prominent projection from the ipsilateral red nucleus via the *central tegmental tract* (Fig. 4C, D, Fig. 5B, C, and Fig. 7A). The inferior olfactory nucleus is the largest of several similar structures located in the rostral medulla. Olivary nuclei also receive input from the cortex, vestibular nuclei, reticular formation, and spinal cord (*spino-olivary tract*). The olfactory nuclei are somatotopically organized and provide a massive projection to the contralateral cerebellum by way of the *inferior cerebellar peduncle* (Fig. 2 and Fig. 4C, D). Olivo-cerebellar and pontocerebellar projections are components of pathways whereby the activities of cortical and subcortical structures can be integrated with cerebellar functions.

8.4. The Tectum of the Midbrain

The tectum of the midbrain includes the *superior colliculus* and the *inferior colliculus*, which are formed by their underlying nuclei (Fig. 2B, Fig. 3, and Fig. 7A, B). The superior colliculus receives input from the optic tract and the visual cortex and contributes fibers to *tectonuclear and tectospinal tracts* (see later). Several nuclei located in the tegmentum at the level of the superior colliculus are the center for vertical gaze (Section 6.3).

A *pretectal area* is located just rostral to the superior colliculus; it receives information about ambient light from both retinas and integrates light reflexes. Pretectal area connections are bilateral, via the *posterior commissure*, to both oculomotor nuclei, which supply parasympathetic innervation to the constrictor of the pupils and the ciliary muscles of the eyes. Other pretectal fibers course through the lateral tegmentum of the brain stem and innervate the sympathetic preganglionic neurons in the upper thoracic spinal cord. Sympathetic postganglionic fibers follow the vasculature to the head and provide innervation to the *dilator of the pupil* and to the *tarsal muscles* of the eyelids.

The inferior colliculus receives auditory input from both cochleae via the lateral lemniscus. Inferior colliculus projects to the superior colliculus and, via the peduncle of the inferior colliculus, to the medial geniculate nucleus of the thalamus (Fig. 2B and Fig. 7A).

Tectonuclear and tectospinal tracts innervate brain stem and spinal cord musculature and thereby provide for reflex orientation of the eyes, head, and upper trunk toward light and sound stimuli. These tracts descend through the brain stem near the midline posterior to the medial lemniscus and continue in the ventral funiculus of the spinal cord.

8.5. Ventral Tegmental Area

The *ventral tegmental area* is located just medial to the substantia nigra (Fig. 7B). These dopaminergic neurons are part of the *mesolimbic and mesocortical projections* to structures such as *nucleus accumbens*, *amygdala*, *cingulate gyrus*, and other forebrain regions. These dopaminergic projections to the *limbic system* participate in the generation of emotion and behavior (see Chapter 11 for details).

8.6. Periaqueductal Gray

The *periaqueductal gray (PAG)* consists of several subgroups of neurons that project to the brain stem and hypothalamus (Fig. 5C and Fig. 7). Many of the PAG neurons express opiate receptors and provide a prominent enkephalinergic projection to the brain stem, especially to the raphe nuclei of the pons and medulla (Fig. 4C, D and Fig. 5A–D). PAG receives input from collateral branches of ascending pain pathway and from the hypothalamus and several limbic structures suggesting a wider role in modulating emotional and autonomic responses to pain (see Chapter 11 and Chapter 21 for details).

8.7. Locus Ceruleus

Locus ceruleus (LC) is a discrete cluster of pigmented *noradrenergic neurons* located in the tegmentum of the rostral pons just anterior to the floor of the fourth ventricle (Fig. 5C; see also Fig. 3 in Chapter 11). Other adrenergic neurons are located in the lateral reticular formation in the medulla (Fig. 4C, D). LC axons traverse the central tegmental tract to distribute to thalamus, hypothalamus, and throughout the cerebral cortex (Fig. 5B, C and Fig. 7A). These adrenergic pathways play a role in generating the sleep cycle and maintaining attention. Descending adrenergic fibers, from LC and from the lateral reticular formation, project to the cerebellum via the superior and inferior cerebellar peduncles, respectively. These cerebellar projections participate in the regulation of posture and muscle tone.

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Disorders of the Autonomic Nervous System

Gregory Cooper, Gerald Eichhorn and Robert Rodnitzky

Clinical symptoms of autonomic dysfunction can arise from abnormalities of autonomic pathways within the central or peripheral nervous systems. Mild autonomic dysfunction can occur in otherwise normal persons with advancing age. More substantial autonomic symptoms arise when there is structural disruption of autonomic pathways or when they are involved in degenerative or metabolic conditions. One of the most disabling symptoms of autonomic dysfunction can be orthostatic hypotension, or a significant drop in blood pressure with assuming an upright posture. Other symptoms of autonomic dysfunction include impaired bladder control, erectile failure, and gastrointestinal dysfunction, manifesting as gastroparesis, constipation, and even diarrhea and occasional incontinence.

PERIPHERAL-NERVE DYSFUNCTION

Nerve fibers subserving autonomic function are easily damaged in diseases of the peripheral nerves. The peripheral neuropathies that are most likely to result in autonomic symptoms are those that cause acute demyelination or involve small myelinated or unmyelinated fibers. Among the most common neuropathies in this category are those caused by diabetes, amyloidosis, porphyria, and the Guillain-Barré syndrome. In a neuropathy such as that associated with Friedreich's ataxia, in which the large fibers are reduced in number but small fibers are preserved, there are virtually no autonomic symptoms. Between these two extremes are a variety of neuropathies in which autonomic symptoms are present but which do not constitute the primary disability. Examples include those caused by alcohol abuse, nutritional

deficiency, leprosy, chronic kidney failure, and acquired immunodeficiency syndrome.

CENTRAL NERVOUS SYSTEM DISORDERS

Abnormalities of the brain or spinal cord can result in autonomic dysfunction. The autonomic dysfunction can be the primary clinical abnormality or part of a wider syndrome affecting many parts of the nervous system. *Pure autonomic failure* is a primary autonomic disorder in which there is gradual development of symptoms such as bladder dysfunction, decreased tear production, erectile dysfunction, reduced sweating, and orthostatic hypotension (e.g., decreased blood pressure on standing). The primary pathologic finding is a loss of sympathetic preganglionic cell bodies in the intermediolateral column of the spinal cord. This same syndrome may occur with more diffuse involvement of the central nervous system (CNS) and other non-autonomic symptoms. When other nervous system structures are involved, such as the cerebellum, the entire conglomerate of autonomic, parkinsonian, and cerebellar symptoms is referred to as *multiple system atrophy (MSA)*. This condition is often divided into three subgroups depending on the predominant associated symptoms. If autonomic failure is associated with prominent signs of Parkinson's disease, it is assumed that nigrostriatal degeneration has also occurred, and the condition is termed *striatonigral degeneration*. When associated with prominent cerebellar symptoms, such as ataxia, this is termed *olivopontocerebellar atrophy (OPCA)*. When autonomic dysfunction is the predominant finding, this is termed *Shy-Drager syndrome*.

Among spinal cord disorders, prominent autonomic dysfunction is most likely to be associated

with severe transverse lesions. Such lesions at or above the midthoracic spinal cord level typically result in severe orthostatic hypotension. In such cases, there is inadequate control of the sympathetic outflow from the spinal cord below the lesion. This results in inadequate reflex constriction of blood vessels in response to the normal drop in blood pressure associated with assuming the standing position. Persons with transverse spinal cord lesions are typically paraplegic and unable to stand on their own, but caution must be exercised in moving them passively to the upright position because of the risk of severe hypotension.

Bladder function can also be affected by a variety of spinal cord lesions. A lesion involving the conus medullaris or the cauda equina results in an autonomic neurogenic bladder characterized by inability to initiate micturition and marked urinary retention. In this circumstance, the afferent or efferent arc of the micturition reflex, both of which travel through cauda equina parasympathetic nerves, or the center for micturition located in the second, third, or fourth segments of the sacral spinal cord, have been involved by the offending lesion. With spinal cord lesions above the sacral parasympathetic center for bladder control, the bladder reflex remains intact but cannot be controlled by descending inhibitory influences from supraspinal centers. In this situation, the voiding reflex goes unchecked, and there is frequent, spontaneous, and precipitous micturition. Patients with spinal cord lesions of this type can be taught to precipitate this reflex voluntarily by stroking the skin in sacral innervated areas or by gently compressing the bladder through the abdomen. Using this technique, they regain some control over the timing of voiding.

ABNORMALITIES OF NEUROTRANSMITTER METABOLISM

A deficiency or an excess of neurotransmitters subserving autonomic function can occur. A deficiency of dopamine β -hydroxylase, the enzyme responsible for the final reaction in the synthesis of norepinephrine, has been documented in some persons and appears to be inherited as an autosomal recessive trait. Marked reduction in plasma and cerebrospinal fluid (CSF) norepinephrine levels can be documented in these patients, who often suffer from symptoms of sympathetic autonomic dysfunction, especially orthostatic hypotension. The opposite circumstance prevails in patients with a catecholamine-producing

tumor of the adrenal gland known as a pheochromocytoma. In this condition, excessive production of norepinephrine results in hypertension.

Plasma norepinephrine levels can be measured. In the case of pheochromocytoma, the levels of norepinephrine and several other catecholamines measured over a 24-h period are typically elevated. Plasma norepinephrine levels can be useful for other purposes. The response of plasma norepinephrine levels to standing has been used as a means of differentiating preganglionic from postganglionic sympathetic failure. Because most plasma norepinephrine is believed to be derived from sympathetic postganglionic nerve terminals, resting levels of plasma norepinephrine are expected to be normal in a preganglionic sympathetic lesion. When these persons stand, the otherwise normal postganglionic neuron cannot be activated to produce the normal rise in norepinephrine with the expected spillover into the plasma. In postganglionic sympathetic lesions, even the resting level is diminished because of inadequate numbers of norepinephrine-releasing nerve terminals. In this circumstance, the elevation in plasma norepinephrine levels after standing is also less than normal, depending on how widespread the postganglionic dysfunction is.

CLINICAL TESTS USEFUL IN DOCUMENTING AUTONOMIC DYSFUNCTION

Recording the blood pressure in the supine and standing positions while determining the concurrent heart rate is a simple and extremely useful test for sympathetic autonomic dysfunction. If after 2 minutes of standing the systolic blood pressure has fallen by 30 mm Hg or more, a diagnosis of orthostatic hypotension is justified. Absence of acceleration in the heart rate in the face of this hypotension further confirms the presence of autonomic dysfunction.

A variety of provocative tests can be performed to assess the sympathetic mechanisms that control heart rate and blood pressure. These include measuring the extent to which a sustained hand grip, immersing a hand in ice water, or performing difficult mental arithmetic stimulate the sympathetic outflow and elevate the heart rate and blood pressure. One mechanism of evaluating parasympathetic control of heart rate is to have the patient briefly execute the Valsalva maneuver (e.g., forcefully attempt to expel breath against a closed glottis). This results in a transient increase in intrathoracic pressure; with resultant decreased cardiac filling and a lowering of blood

pressure. The normal baroreflex response to lower blood pressure should be an elevation in heart rate. On release, there should be an overshoot of the normal blood pressure and slowing of the heart rate. Absence of these reactions is a sign of parasympathetic dysfunction. The response to the Valsalva maneuver is actually much more complex than this and can be divided into three or four component phases, but in its simplest form, it can be a useful screening test for parasympathetic function.

Sweating or sudomotor function is a sympathetic function that can be evaluated by applying Alizarin-red powder to the skin. When the body temperature is elevated, the powder, which is initially white, becomes red wherever it is in contact with perspiration. This can be particularly striking in conditions such as loss of sweating (e.g., anhidrosis) on one side of the face.

Extremely localized autonomic functions can be measured. A classic example is the assessment of sympathetic innervation of the pupil and eyelid. When this innervation is interrupted by a lesion in the sympathetic chain, a syndrome consisting of ptosis (e.g., drooping) of the eyelid, miosis (e.g., a smaller than normal pupil), and anhidrosis on the same side of the face may occur. Collectively, these neurologic signs are known as *Horner's syndrome*. A series of pharmacologic tests can determine whether Horner's syndrome is present, and if so, whether it is caused by involvement of the first- or second-order neuron in the sympathetic chain (i.e., preganglionic lesion) or the third-order neuron (e.g., postganglionic lesion). An initial test that can determine if Horner's syndrome is present involves the instillation of a dilute

solution of cocaine into the conjunctival sac of the involved eye and the opposite normal eye. Cocaine inhibits re-uptake of synaptic norepinephrine. In the normal eye, this causes enhanced sympathetic stimulation of the iris dilator muscle, resulting in enlargement of the pupil. On the side with sympathetic dysfunction, there has been little or no synaptic norepinephrine elaborated, and the pupil dilates considerably less than that on the normal side. This effect occurs regardless of the location of the lesion within the sympathetic pathway.

The next step differentiates a preganglionic lesion from a postganglionic lesion. Paredrine, an amphetamine derivative, is instilled into both eyes. Because amphetamine enhances the release of norepinephrine from sympathetic terminals, the expected normal response is pupillary dilation, but this only occurs if there is a healthy third-order (postganglionic) neuron. A normal response to Paredrine in a patient with Horner's syndrome suggests that the causative sympathetic lesion is located in the first- or second-order neuron. An abnormal response to Paredrine indicates that the pathology is in the postganglionic neuron.

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Harold H. Traurig

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1. INTRODUCTION

The reticular formation is an aggregation of several subtypes of interconnected neurons extending throughout the brain stem tegmentum. It is continuous and functionally related to the hypothalamus and midline and interlaminar nuclei of the dorsal thalamus. The reticular pathways integrate sensory, visceral, limbic, and motor functions. Reticular circuits project throughout the central nervous system and exert important influences on autonomic regulation of vital organ systems, behavior, somatic motor activities, sleep cycles, alertness, and pain modulation.

The term *reticular formation* was adopted by early anatomists to distinguish what appeared to them as diffusely interconnected neurons in the brain stem tegmentum compared with the anatomically more distinct nuclei associated with the cranial nerves. This characterization of the reticular formation suggested that it was poorly organized and served only primitive functions. However, some early investigators, notably Cajal, recognized organization and specificity in the reticular formation based on neuron morphology, location in the brain stem, and connectivity. Despite Cajal's work, the reticular formation evoked little research interest until the electrophysiologic studies by Moruzzi and Magoun in the 1940s and 1950s demonstrated that the maintenance of

consciousness and alertness depended on input from sensory pathways traversing the brain stem reticular formation to the thalamus and cerebral cortex.

Later studies demonstrate that inputs to specific components of the reticular formation originate from cerebral cortex, striatum, limbic structures, hypothalamus, cerebellum, and the spinal cord. Certain components of the reticular formation project to these same structures, often through the thalamus or hypothalamus, as well as to the somatic and visceral motor nuclei of the brain stem and spinal cord.

Additionally, it is evident from numerous recent research reports that subgroups of reticular neurons and nerve terminals contain specific combinations of transmitters and peptides, such as serotonin, norepinephrine, acetylcholine, dopamine, glutamate, gamma-aminobutyric acid (GABA), and enkephalin, suggesting that reticular formation functions also depend on complex neurochemical interactions.

Current evidence reveals that the reticular formation provides an important matrix for neural integration. Ascending reticular formation projections play key roles in alertness, behavior, and affect. Brain stem reticular formation circuits, in concert with limbic and hypothalamic inputs, regulate cardiovascular and respiratory rhythms and other visceral responses through influences on cranial nerve nuclei and descending connections with autonomic centers in the spinal cord. Reticulospinal tracts influence transmission of sensory modalities by dorsal horn neurons and activities of

alpha and gamma motor neurons thereby modulating pain transmission, skeletal muscle tone, and somatic reflexes.

2. ANATOMIC CHARACTERISTICS OF RETICULAR FORMATION NEURONS

The reticular formation is subdivided into a number of nuclei based on their anatomic locations. The more prominent of the reticular nuclei are described here. It is convenient to characterize the reticular formation as three columns of neurons extending throughout most of the brain stem tegmentum (Fig. 1). Although these columns of neurons are not separated by clearly defined anatomic boundaries and often overlap one another, they are distinct from one another based on neuron morphology, neurochemical phenotype, circuitry, and location in the mediolateral plane. The unpaired median column or *raphe nuclei* are located along the midline of the brain stem tegmentum (Fig. 1; *see also* Fig. 3). (Raphe means “seam” and refers to the midline of the brain stem.) The paired medial (central) and lateral columns of nuclei are found in the central (immediately lateral to the midline) and lateral portions of the tegmentum, respectively (Fig. 1). The median and medial columns are the *magnocellular regions*, whereas the lateral column is the *parvocellular region*; these terms reflect the large and small size of the majority of the neurons in these columns, respectively.

Reticular formation neurons possess an aggregate of characteristics that distinguish them from most other brain stem neurons. Many have extensive dendritic arborizations oriented in a plane perpendicular to the long axis of the brain stem and to the ascending sensory and descending motor tracts. Other brain stem neurons have restricted dendritic fields. Reticular formation neuron dendrites typically intermingle with the axons of motor and sensory tracts suggesting functional interactions. Axons of many reticular formation neurons are long, form numerous collaterals, and have ascending and descending branches. Therefore, a single reticular neuron could influence brain stem and spinal cord neurons associated with several different somatic or visceral functions. Inputs to reticular neurons typically originate from many and varied sources. Taken together, these anatomic arrangements reflect the modulatory and integrative roles of the reticular formation.

2.1. Raphe (Median Column) of the Reticular Formation

Several subgroups of ascending and decussating axons traverse the raphe of the brain stem; the more prominent of these are the medial lemniscus, olivocerebellar tract, olivocerebellar tract, and trapezoid body (fibers). Raphe neurons are scattered among these traversing axons most prominently in the medulla and pons. Raphe neurons also extend into the midbrain (squares in Fig. 1; Fig. 2). The following raphe nuclei have been described based on neuron morphology: *raphe obscurus*, *raphe pallidus*, and *raphe magnus* in the medulla (Fig. 1A, B); *raphe magnus*, *raphe pontis*, and *superior central* in the pons (Fig. 1C, D); *dorsal tegmental nucleus* and *nucleus linearis* (Fig. 1E, F) in the midbrain. Many of the raphe neurons contain *serotonin (5-hydroxytryptamine)* and use this *indoleamine* as a transmitter in chemical transmission (Fig. 2).

Raphe serotonergic neurons of the caudal brain stem, such as *raphe magnus*, *raphe pallidus*, and *raphe obscurus*, project prominently to the spinal cord (*see* Fig. 4). Available evidence implies that serotonergic terminals ending in substantia gelatinosa of the dorsal horn play a key role in modulating pain transmission to conscious centers.

Many pontine and midbrain serotonergic raphe neurons, such as *raphe pontis*, *superior central*, and *dorsal tegmental nucleus*, have direct and indirect rostral projections through the diencephalon to amygdala, forebrain, and cerebral cortex. They also project to the noradrenergic neurons of the *locus coeruleus* (Fig. 3 and Fig. 4). These ascending circuits participate in the regulation of alertness and sleep cycles.

2.2. Medial “Effector” Columns of the Reticular Formation

The bilateral medial (central) columns of the reticular formation occupy the central portion of the medullary and pontine tegmentum just lateral to the raphe nuclei (diamonds in Fig. 1). The medial columns are subdivided into the *central reticular* (Fig. 1A, B), *gigantocellular nuclei* in the medulla, *caudal and rostral (oral) pontine reticular nuclei* in the pons (Fig. 1C, D), and rostral pontine reticular nucleus in the midbrain (Fig. 1E). Most of these neurons are large (magnocellular) and have extensive dendritic arborizations oriented perpendicular to the long axis of the brain stem. Long ascending and descending axons, with numerous collateral branches, originate from medial column neurons. These terminate in other regions of the reticular

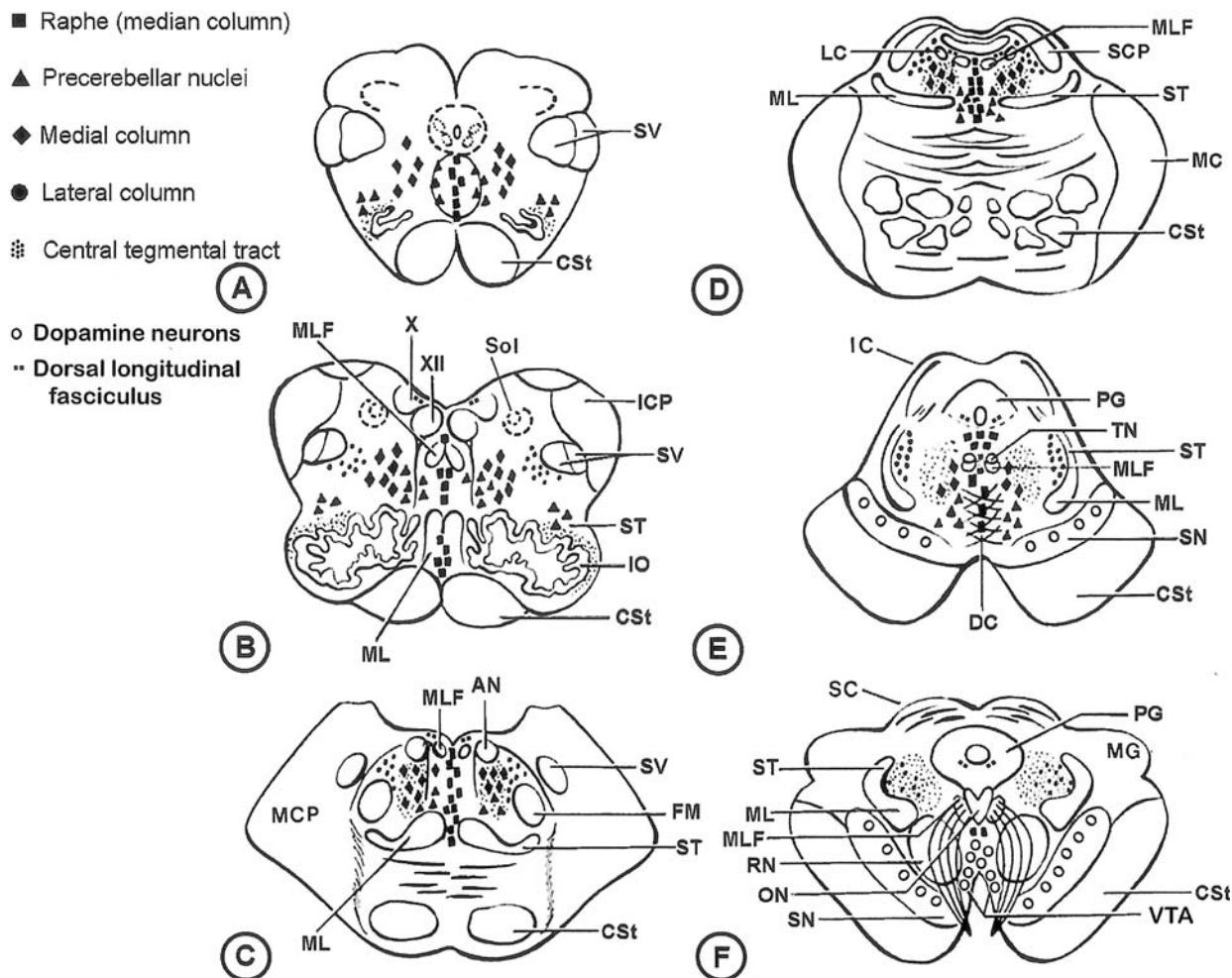


Fig. 1. Representations of the major nuclei of the reticular formation in the brain stem. Anatomic boundaries of these nuclei are indistinct. Numerous subnuclei have been identified based on neuron morphology and neurochemical characteristics. **(A)** Caudal medulla: level of the sensory decussation (medial lemniscus). **(B)** Mid-medulla: level of the vagal and hypoglossal nuclei. **(C)** Caudal pons: level of the facial and abducens nuclei. **(D)** Rostral pons: level of the locus coeruleus. **(E)** Midbrain: level of the inferior colliculus. **(F)** Midbrain: level of the superior colliculus. *Median column or raphe of reticular nuclei* (squares) includes nucleus raphe obscurus and nucleus raphe pallidus (medulla, **A** and **B**), nucleus raphe magnus, nucleus raphe pontis, and superior central nucleus (pons, **C** and **D**), and nucleus linearis and dorsal tegmental nucleus (midbrain, **E** and **F**). *Medial (central) column of reticular nuclei* (diamonds) includes central reticular nucleus and gigantocellular nucleus (medulla, **A** and **B**), caudal and rostral pontine reticular nuclei (pons, **C** and **D**), and rostral pontine reticular nucleus (midbrain, **E**). *Lateral column of reticular nuclei* (large dots) includes ventrolateral reticular nucleus in the parvocellular region (medulla and pons, **B** and **C**), parabrachial nucleus (pons **D** and **E**), and pedunculopontine nucleus and cuneiform nucleus (midbrain, **E** and **F**). *Precerebellar nuclei* (triangles) include paramedian reticular nuclei (medulla and pons, **A**, **B**, and **C**), lateral reticular nucleus (medulla, **A** and **B**), and pontine reticulotegmental nuclei (**D** and **E**). Stippling represents the ascending and descending fibers of the central tegmental tract. Open circles indicate the dopaminergic neurons of the substantia nigra (SN) and the ventral tegmental (mesolimbic) region (VTR). Small squares locate the dorsal longitudinal fasciculus ventral to the cerebral aqueduct and fourth ventricle. Other relevant structures: AN, abducens nucleus; CSt, corticospinal tract; DC, decussation of superior cerebellar peduncle; FM, facial motor nucleus; IC, inferior colliculus; ICP, inferior cerebellar peduncle; IO, inferior olive nucleus; LC, locus coeruleus; MCP, middle cerebellar peduncle; MG, medial geniculate nucleus; ML, medial lemniscus; MLF, medial longitudinal fasciculus; ON, oculomotor nucleus; PG, periaqueductal gray; RN, red nucleus; SC, superior colliculus; SCP, superior cerebellar peduncle; Sol, solitary nucleus; SN, substantia nigra; ST, spinothalamic (anterolateral) tracts; SV, spinal nucleus and tract of the trigeminal; TN, trochlear nucleus; X, dorsal motor nucleus of the vagus; XII, hypoglossal nucleus.

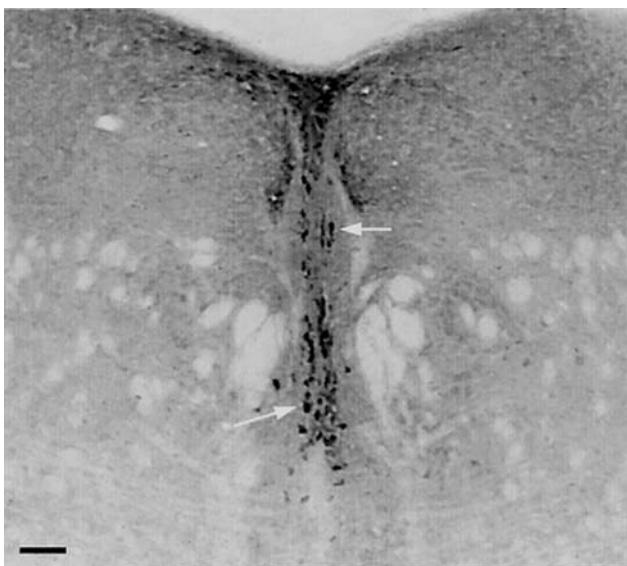


Fig. 2. Serotonergic neurons of the raphe. Immunocytochemical localization of a marker for serotonin in the cell bodies of neurons (arrows) in the brain stem raphe of the mouse. Bar = 100 μ m. (Illustration courtesy of Dr. Melissa Zwick, Richard Stockton College.)



Fig. 3. Locus coeruleus, raphe and central tegmental tract. Unstained section of human rostral pons demonstrating the pigmented, noradrenergic neurons of the locus coeruleus (arrow). The shaft of the arrow overlies the central tegmental tract. Arrowheads indicate the raphe region of the pons. Bar = 5 mm.

formation, limbic structures, hypothalamus, thalamus (e.g., interlaminar nuclei), cranial nerve nuclei, and the spinal cord (Fig. 4 and Fig. 5). The ascending and descending axons of the medial columns, or “effector” neurons, collect as *reticulocortical*, *reticulonuclear*, *reticulohypothalamic*, and *reticulospinal tracts* and convey reticular formation influences throughout the central nervous system.

2.3. Lateral “Afferent” Columns of the Reticular Formation

The principal lateral column nuclei (dots in Fig. 1) are composed mostly of small (parvocellular) neurons and include, the *parvocellular nucleus (region)* and the *ventrolateral nucleus* in the lateral tegmentum of the medulla and pons (Fig. 1B, C), *parabrachial nucleus* in the pons (Fig. 1D), and *pedunculopontine* and *cuneiform nuclei* in the midbrain (Fig. 1F). Lateral column (parvocellular) components in the medulla are located between the medial reticular column medially and the spinal trigeminal nucleus laterally. Rostrally, the lateral column is ventromedial to the superior cerebellar peduncle in the pons and medial to the medial lemniscus and anterolateral tracts in the midbrain.

The parvocellular neurons of the lateral columns are characterized by prominent afferent projections from axon collaterals of most sensory tracts. The sensory projections received by the lateral column neurons are relayed medially to the medial column; the medial column nuclei have reciprocal connections with the raphe nuclei (Fig. 5). For example, the parvocellular neurons in the lateral column in the medulla and pons relay somatic and visceral sensory information from the ascending anterolateral, spinoreticular, and trigeminothalamic tracts to the medial column. Other visceral afferents are conveyed by the facial, glossopharyngeal, and vagus nerves to the *solitary nucleus* in the medulla (Fig. 1B). Solitary neurons relay integrated visceral sensory information to medial and lateral (parvocellular) columns of the reticular formation. Finally, cerebellar, auditory, visual, and vestibular afferents also terminate in the medial and lateral (parvocellular) columns by direct and indirect pathways (Fig. 5).

Functionally, the ascending and descending reticular tracts provide regulatory circuits that modulate many important functions and reflexes (Fig. 4 and Fig. 5). For example, many of these terminals modulate nociceptive transmissions by activating serotonergic raphe nuclei, which, in turn, activate enkephalinergic interneurons in the substantia gelatinosa of the dorsal horn and in the spinal trigeminal nucleus (see Section 5.3 for details). They also activate ascending pathways that follow the *central tegmental tract* and relay in the thalamus. Related thalamocortical fibers project throughout the cerebral cortex to facilitate alertness (Fig. 4; see Section 5.1). Connections of the parabrachial nucleus in the pons and midbrain suggest roles in visceral and limbic functions. The *pedunculopontine* and *cuneiform nuclei* are involved in motor functions based on their projections

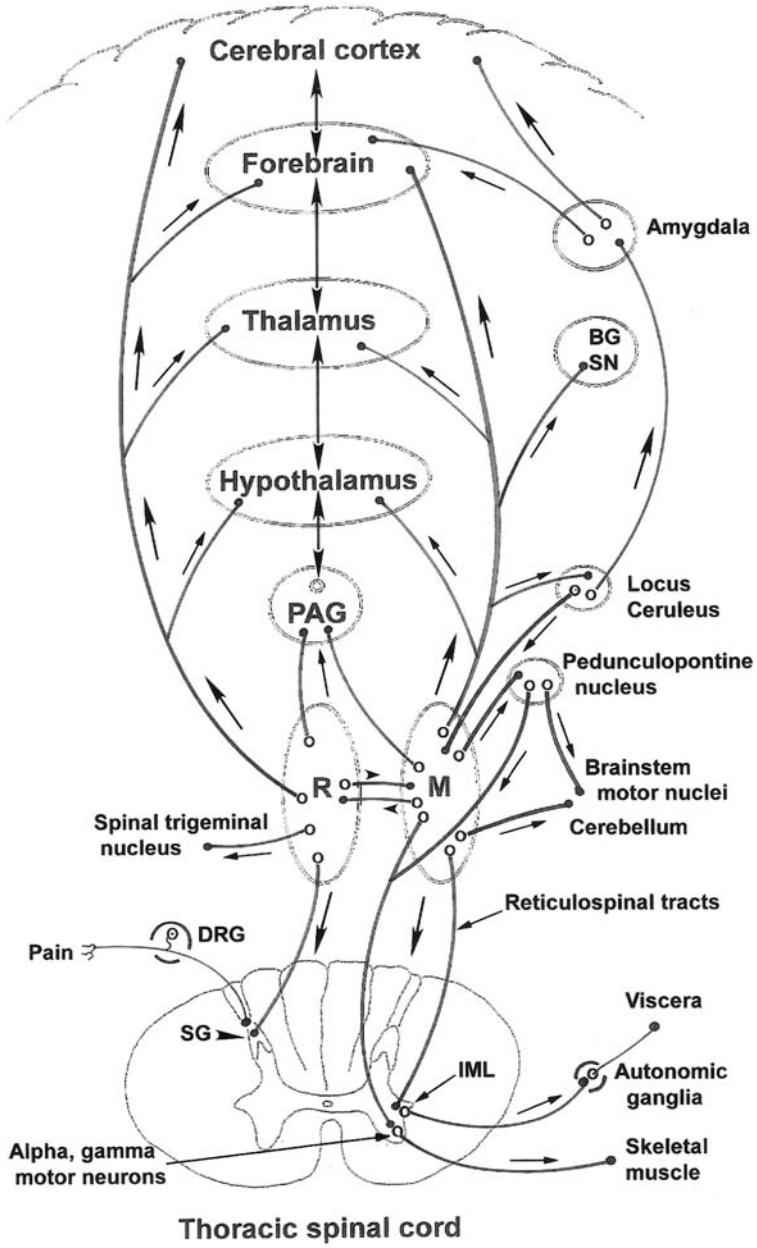


Fig. 4. *Bold arrows and arrowheads represent the principal efferent projections of the reticular formation. Neuron cell bodies and terminals are represented by open circles and black dots, respectively. Reticular efferent projections to rostral targets follow the medial forebrain bundle, mamillotegmental tract, dorsal longitudinal fasciculus, central tegmental tract, and other pathways. R, raphe (median column) neurons; M, medial (central) column neurons. Lateral column neurons are represented by the pedunculopontine nucleus. BG, basal ganglia; DRG, dorsal root ganglion; IML, intermediolateral nucleus; PAG, periaqueductal gray; SG, substantia gelatinosa of the dorsal horn; SN, substantia nigra.*

to the motor cortex, basal ganglia, substantia nigra, precerebellar, and raphe nuclei (see Section 5.4).

3. RETICULAR FORMATION PATHWAYS

Ascending and descending pathways in the reticular formation are diffuse and multisynaptic. Most efferent projections originate from the magnocellular

nuclei (Fig. 4). Afferent projections to the reticular formation include those from the spinal cord, solitary nucleus, cerebellum, periaqueductal gray, hypothalamus, thalamus, areas of the cerebral cortex, and directly and indirectly from limbic structures (Fig. 5). A few afferent and efferent tracts are described in this section; others are described with their special functions in the following sections of this chapter.

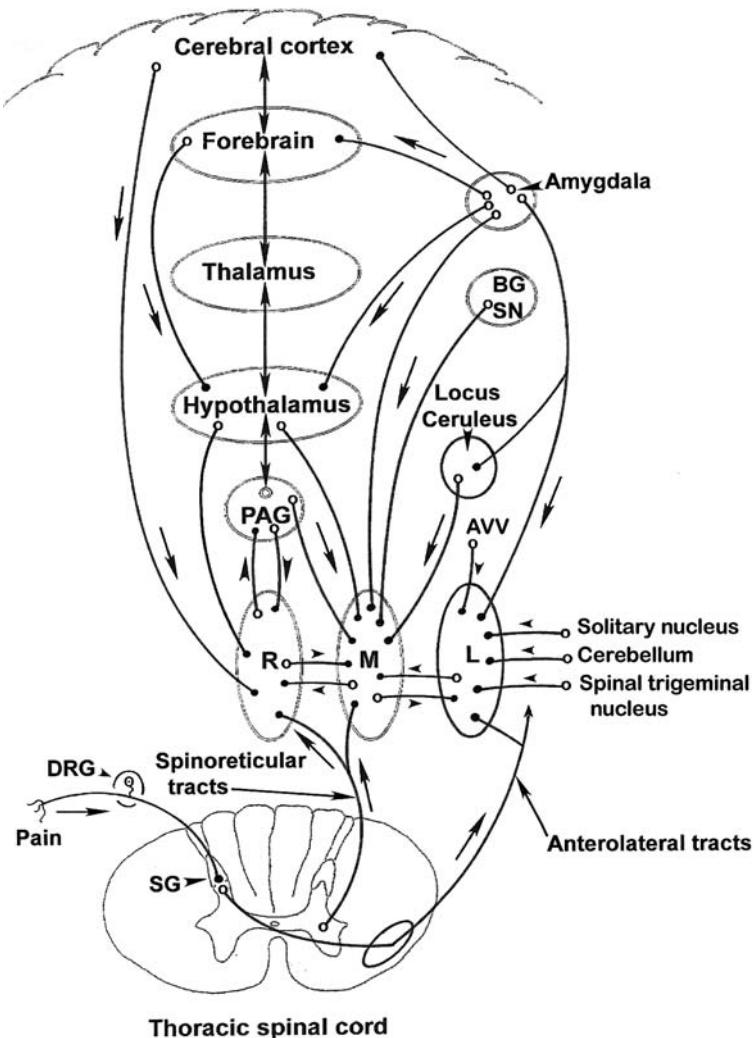


Fig. 5. Bold arrows and arrowheads represent the principal afferent projections to the reticular formation. Neuron cell bodies and terminals are represented by open circles and black dots, respectively. Afferent projections from rostral sources follow medial forebrain bundle, mammillotegmental tract, mammillary peduncle, dorsal longitudinal fasciculus, central tegmental tract, and other pathways to the reticular formation. R, raphe (median column) neurons; M, medial (central) column neurons; L, lateral column (parvocellular) neurons. AVV, afferents from the auditory, vestibular, and visual systems; BG, basal ganglia; DRG, dorsal root ganglia; PAG, periaqueductal gray; SG, substantia gelatinosa of the dorsal horn, SN, substantia nigra.

3.1. Reticulospinal Tracts

The *lateral and medial reticulospinal tracts* arise from the magnocellular neurons of the medial column in the medulla and pons, respectively (Fig. 4). The *medial reticulospinal tract* originates mostly from the ipsilateral *pontine reticular nucleus* and projects to the spinal cord via the ventral funiculus. *Gigantocellular nucleus* in the medulla projects mostly ipsilaterally as the *lateral reticulospinal tract* located in the ventral part of the lateral funiculus of the spinal cord. Both of these projections provide complex regulation of *alpha and gamma motor neurons*.

Other reticulospinal fibers originate from the *lateral tegmental nucleus* located between the solitary and the spinal trigeminal nuclei in the medullary tegmentum. These *cholinergic neurons* project to the *intermediolateral nucleus* in the lateral horn of the thoracic and upper lumbar spinal cord and innervate *sympathetic preganglionic neurons* (Fig. 4). In addition, some reticulospinal fibers terminate on *parasympathetic preganglionic nuclei* and somatic motor neurons in the sacral spinal cord (S_2, S_3, S_4). These projections provide critical links between visceral regulatory centers in the hypothalamus, reticular formation, and autonomic neurons located in the brain stem and spinal cord.

3.2. Spinoreticular Tracts

Visceral sensory fibers return to the central nervous system from most viscera and the vasculature. These modalities include visceral pain and sensory information related to visceral reflexes (e.g., gag reflex) and functions (e.g., micturition) (Fig. 5). The primary sensory fibers follow the sympathetic and parasympathetic nerves and have their cell bodies in dorsal root ganglia or in the sensory ganglia associated with the facial, glossopharyngeal, and vagus nerves.

In the spinal cord, the visceral sensory fibers terminate in the dorsal horn, in the brain stem, most terminate in the solitary nucleus (Fig. 1B). Secondary neurons provide reflex connections with autonomic neurons in the spinal cord and brain stem. In the spinal cord, other secondary fibers form bilateral *spinoreticular tracts* that convey visceral sensations to the reticular formation and indirectly to the periventricular gray, hypothalamus, and thalamus. Some crossed secondary fibers join the anterolateral system and convey visceral sensations to the thalamus. Thalamocortical fibers project to the post central gyrus and the cortex of the insula where visceral pain and other visceral sensations reach consciousness.

3.3. Central Tegmental Tract

The *central tegmental tract* consists of afferent projections to the reticular nuclei and ascending and descending connections between reticular nuclei and other functionally related structures. It extends from midbrain levels to the caudal medulla and is most distinct at caudal midbrain and rostral pontine levels (Fig. 1A–F and Fig. 3). Ascending central tegmental projections arise from *noradrenergic neurons* of the *locus coeruleus* and from *cholinergic neurons* of the *pedunculopontine nucleus* and project to the interlaminar thalamic nucleus, forebrain, and cerebral cortex (Fig. 4). These projections play a key role in maintaining consciousness and in regulating the sleep-wake cycle. Most descending fibers terminate in the *inferior olfactory nucleus* in the medulla and participate in regulation of motor activities (Fig. 1A, B).

3.4. Mammillotegmental Tract and Mammillary Peduncle

Neurons in posterior regions of the hypothalamus, including the mammillary bodies, form the *mammillary fasciculus*. The descending branch of

the mammillary fasciculus projects to the brain stem as the *mammillotegmental tract*. It terminates in the periaqueductal gray and among the nuclei of the midbrain reticular formation. Therefore, the mammillotegmental tract forms an indirect link between the visceral regulatory centers in the posterior hypothalamus through the reticular formation to visceral reflex centers and autonomic nuclei in the brain stem and spinal cord. The *mammillary peduncle* consists mostly of ascending fibers (reticulohypothalamic tract) from the midbrain reticular formation to the posterior hypothalamus (Fig. 4 and Fig. 5).

3.5. Dorsal Longitudinal Fasciculus

Neurons in the periventricular and other regions of the hypothalamus project to the periaqueductal gray and the rostral reticular formation as the *dorsal longitudinal fasciculus*. It is a diffuse collection of small fibers located in the periaqueductal gray just anterior to the aqueduct and fourth ventricle (small squares in Fig. 1B–F). As described for the mammillotegmental tract, the dorsal longitudinal fasciculus provides an indirect link, through the reticular formation, between the hypothalamus and visceral reflex and autonomic centers in the brain stem and spinal cord.

3.6. Medial Forebrain Bundle

The *medial forebrain bundle* contains ascending and descending fibers that course through the lateral hypothalamus making synaptic contact with various hypothalamic nuclei. It connects limbic forebrain structures and lateral hypothalamic nuclei with the rostral reticular formation and periaqueductal gray. The *amygdala*, particularly its central nucleus, projects via the medial forebrain bundle to the midbrain reticular formation (Fig. 4).

3.7. Direct Pathways to Brain Stem and Spinal Cord Autonomic Nuclei

The hypothalamus projects *direct hypothalamomedullary and hypothalamospinal* fibers to the brain stem and spinal cord autonomic nuclei. Many of these descending fibers follow the reticular formation pathways described above and traverse the brain stem reticular formation to reach their targets. The hypothalamospinal fibers course through the lateral funiculus of the spinal cord to reach their targets, the preganglionic sympathetic neurons in the

intermediolateral nucleus in the lateral horn at thoracic and lumbar levels, and the preganglionic parasympathetic nuclei at sacral levels.

4. OTHERS RELATED BRAIN STEM NUCLEI

Other named brain stem nuclei are functionally closely related to the reticular formation. They are briefly described below and considered in detail in other chapters.

4.1. Substantia Nigra and Basal Ganglia

The *substantia nigra* of the midbrain consists of *compact and reticular regions*. Neurons of the compact portion express the catecholamine neurotransmitter *dopamine* (Fig. 1E, F). The dopaminergic compact region neurons project to the striatum (putamen and caudate nucleus). In the striatum, dopamine has both facilitatory and inhibitory effects depending on the receptor type expressed by striatal neurons. The dopaminergic projection to the striatum plays a key role in regulating the posture and muscle tone associated with voluntary movements. Degeneration of the dopaminergic substantia nigra neurons results in *Parkinson's disease*.

The substantia nigra reticular region receives *GABAergic* and *substance P* projections from the striatum and provides GABAergic innervation to the substantia nigra dopaminergic neurons, the reticular formation, and ventral anterior and ventral lateral nuclei of the thalamus. The basal ganglia and the substantia nigra have reciprocal connections with the midbrain and rostral pontine reticular formation, specifically with the serotonergic rostral raphe nuclei and the cholinergic pedunculopontine nucleus (Fig. 4 and Fig. 5). These circuits provide important functional links between the motor regulatory centers in the motor cortex and basal ganglia and the reticular formation. Reticulonuclear and reticulospinal tracts project reticular formation regulation to the alpha and gamma motor neurons associated with the skeletal muscles innervated by the cranial and spinal nerves.

4.2. Red Nucleus

The *red nucleus* occupies the central midbrain tegmentum (Fig. 1F). Its functional role is exclusively related to contralateral motor regulation. The red nucleus receives inputs predominately from the ipsilateral motor cortex, spinal cord, and contralateral cerebellum. In addition, it is reciprocally connected with the lateral reticular nucleus in the medulla, which also projects to the cerebellum. It gives rise to

contralateral *rubronuclear* and *rubrospinal tracts* that terminate on brain stem reticular neurons, cranial nerve motor nuclei, and the motor neurons of the rostral portion of the spinal cord. Another major projection of the red nucleus follows the *central tegmental tract* (stippling in Fig. 1) to the ipsilateral *inferior olfactory nucleus* (Fig. 1A, B).

4.3. Precerebellar Nuclei

Three reticular formation nuclei in the caudal brain stem, collectively termed the *precerebellar reticular nuclei*, have restricted functional roles related to the motor system because they project almost exclusively to the cerebellum (triangles in Fig. 1). Two of these nuclei, the *paramedian reticular* (Fig. 1A–C) and the *pontine reticulotegmental nuclei* (Fig. 1D, E), form a parallel column of neurons located between the medial column of the reticular formation and the brain stem raphe. The third precerebellar reticular nucleus, the *lateral reticular nucleus* (Fig. 1A, B), is a conspicuous cluster of large neurons in the lateral tegmentum of the medulla lying between the spinal trigeminal system and the inferior olfactory nucleus. Together, these precerebellar nuclei provide integrating circuits between cerebellum, red nucleus, inferior olfactory nucleus, and reticular formation that participate in regulating motor functions such as posture, equilibrium, and muscle tone.

4.4. Inferior Olivary Nucleus

The *inferior olivary nucleus* (or complex) is a prominent structure in the ventral tegmentum of the medulla. It includes two smaller components, the dorsal and medial accessory olfactory nuclei. The inferior olivary nucleus has connections similar to those of the precerebellar nuclei of the reticular formation. It receives input from the contralateral spinal cord (spino-olivary tract), indirect input from the reticular formation, and a large direct input from the ipsilateral red nucleus by way of the central tegmental tract (Fig. 1 and Fig. 3). Olivocerebellar fibers cross the midline through the medullary reticular formation and enter the cerebellum via the *inferior cerebellar peduncle*. Virtually all olivocerebellar fibers terminate as climbing fibers on *Purkinje neuron* dendrites in the contralateral cerebellar cortex. The cerebellum projects prominently to the contralateral red nucleus via the *dentatorubrothalamic tract*. Therefore, a cerebellum-red nucleus-inferior olivary nucleus-cerebellum circuit is established that plays a key role in regulation of motor activities.

4.5. Periaqueductal Gray

The *periaqueductal gray* surrounds the cerebral aqueduct and consists of numerous axonal terminals and subgroups of neurons containing many different transmitters and peptides (Fig. 1E, F). Many of these neurons have extensive dendrites that intermingle with the midbrain reticular formation. Periaqueductal gray receives its most prominent inputs from the hypothalamus, forebrain, and limbic structures (Fig. 5). Afferents also originate from the parabrachial and other reticular nuclei, the solitary nucleus, and the ascending sensory pathways. Periaqueductal gray provides reciprocal projections to all of the above structures and to the serotonergic raphe nuclei of the brain stem (Fig. 4). Specifically, periaqueductal gray projects to the nucleus raphe magnus in the pons and medulla (Fig. 1C). Nucleus raphe magnus projects serotonergic terminals to the substantia gelatinosa of the spinal cord and to the trigeminal spinal nucleus where the synaptic release of serotonin activates enkephalergic interneurons. The enkephalergic neurons modulate nociceptive stimuli by diminishing activities in the transmission neurons whose axons form the spinothalamic and trigeminothalamic tracts. Thus, the periaqueductal gray, in concert with the raphe nuclei of the reticular formation, plays a key role in integrating limbic and autonomic functions and in modulating pain perceptions (see Chapter 21).

4.6. Ventral Tegmental Region

The *ventral tegmental region* or *mesolimbic region of Tsai* is located in the posterior perforated substance of the ventromedial aspect of the midbrain (Fig. 1F). It receives serotonergic terminals from the rostral raphe nuclei as well as input from other reticular neurons. The ventral tegmental region receives indirect input from the ascending sensory tracts through the reticular formation. The ventral tegmental neurons are dopaminergic and project to *medio-basal frontal cortex, cingulate gyrus, hippocampus, amygdala, nucleus accumbens, basal nucleus of Meynert*, and other limbic and cortical regions.

Accumulating evidence implies that the *mesolimbic* and *mesocortical* dopaminergic projections from the ventral tegmental region play key roles in *alertness, emotion, and cognition*. Schizophrenic symptoms may result from inappropriate activation of the mesolimbic and mesocortical dopamine projections to the forebrain and other limbic structures. In addition, evidence suggests that drugs of abuse activate the mesolimbic and mesocortical projections to those

limbic structures that generate feelings of pleasure and reward. Because activities of some dopaminergic neurons are facilitated by serotonin, it is likely that serotonergic raphe neurons are also an important component of the circuitry that regulates limbic system functions. Available evidence suggests that the development of *schizophrenia* involves other factors such as multiple dopamine receptors and alterations in responsiveness of mesolimbic and/or mesocortical dopamine targets.

4.7. Locus Coeruleus

The *locus coeruleus* is a compact group of pigmented neurons located in the rostral pons ventrolateral to the fourth ventricle and dorsolateral to the *pontine reticular nucleus* (Fig. 1D and Fig. 3). Locus coeruleus neurons express *norepinephrine*, a catecholamine transmitter. Afferents to locus coeruleus arise from the rostral serotonergic raphe nuclei and the cholinergic pedunculopontine nucleus in the lateral tegmentum of the midbrain (Fig. 4). One subgroup of these afferents exerts inhibitory influences, another facilitates locus coeruleus activity. Other locus coeruleus afferents arise from the cerebral cortex, amygdala, hippocampus, periaqueductal gray, hypothalamus, and from limbic structures especially the forebrain. The major efferents of the locus coeruleus are to these same structures and to other reticular formation nuclei (Fig. 5).

These circuits allow the locus coeruleus to respond to new sensory stimuli and, through its widely disseminated noradrenergic projections, exert an alerting and activating influence throughout the brain. In addition, norepinephrine released from the locus coeruleus plays a role in the sleep-wake cycle.

Depression is thought to involve diminished norepinephrine release by locus coeruleus and other neurons. However, current evidence infers that the neurochemical basis for depression is complex and very likely involves other transmitter systems, such as serotonin.

5. RETICULAR FORMATION FUNCTIONS AND INTERACTIONS

Reticular formation morphology, circuitry, and chemical transmission characteristics provide an important matrix for integration of many neural functions. The control of vital functions, such as regulation of blood pressure, depends on integrated circuitry between cortex, diencephalon, reticular formation, spinal cord, and peripheral tissues. To that end,

inputs converge on the reticular formation from almost all somatic and visceral sensory pathways and from cortex, hypothalamus, striatum, limbic structures, and spinal cord (Fig. 5). Moreover, the reticular formation provides prominent projections to these same structures (Fig. 4). The reticular formation also plays key roles in alertness, sleep, control of posture and muscle tone, and in pain modulation.

5.1. The Alerting Response

Early studies demonstrated that stimulation of the reticular formation evokes changes in cortical activity characteristic of the arousal induced by sensory stimulation. Later studies revealed that the ascending spinothalamic and trigeminothalamic tracts provide collateral input to the lateral columns of the reticular formation, for example, the parvocellular nucleus in the pons (Fig. 1C–E). The lateral column neurons project to the medial columns in the medulla and pons, specifically the central (ventral) reticular, gigantocellular, and pontine reticular nuclei (Fig. 1A–D). The medial columns form prominent ascending projections that follow the *central tegmental tract* and terminate in the adrenergic locus coeruleus, cholinergic pedunculopontine nucleus, hypothalamus, and the intralaminar nuclei of the dorsal thalamus (Fig. 3 and Fig. 4; *see also* stippling in Fig. 1). Related thalamocortical fibers relay activation throughout the limbic system and cerebral cortex. Additionally, activation of the locus coeruleus noradrenergic projections to the cortex facilitates the attentional state. Collectively, these rostral projections constitute the *ascending reticular activating system (ARAS)* that supports several important functions. The flow of sensory stimuli through ARAS activates the hypothalamic and limbic structures that regulate emotional and behavioral responses (e.g., responses to pain). More important, the flow of sensory stimuli facilitates cortical activity. For example, activation of the widely disseminated locus coeruleus noradrenergic projections to the cortex is important in facilitating the attentional state and in generating sleep/wake cycles (Fig. 5). Other examples of ARAS activities include the alerting responses to a sudden loud sound, a flash of light, smelling salts, or a splash of cold water in the face. Without cortical activation by ARAS, the individual is less able to detect new specific stimuli, and the level of consciousness is diminished.

There are important clinical implications related to ARAS function. The reticular formation projections in the ARAS traverse the midbrain tegmentum; some

of these projections follow the central tegmental tract (Fig. 1 and Fig. 3). Lesions of the midbrain can interrupt the ARAS leading to *altered levels of consciousness or coma* due to the diminished facilitation of limbic and cortical neurons. Lesions frequently affecting the midbrain include cerebrovascular accidents (i.e., stroke) and head trauma. Cerebrovascular accidents can interfere with the blood supply to the brain stem and therefore alter consciousness because of diminished oxygen supply to reticular neurons and ascending pathways.

This discussion should not suggest that the reticular formation is the “center for consciousness.” Experimental and clinical observations imply that consciousness, which is a person’s ability to be aware of self and environment and to orient toward new stimuli, results from the integrated actions of a number of neural structures including the reticular formation. In that regard, positron emission tomography (PET) scan and functional magnetic resonance imaging (fMRI) studies in humans reveal that metabolic activities are depressed in the reticular formation of the rostral pons and midbrain, the periaqueductal gray, and the thalamus as alertness and consciousness are lost after administration of anesthetic drugs.

Head trauma can induce increased intracranial pressure due to collection of blood (i.e., hematoma) between the skull and the brain or the accumulation of edema fluid in the injured brain. Because the brain is encased in the skull, the increased intracranial pressure causes the medial aspect of the temporal lobe, specifically the *uncus of the parahippocampal gyrus*, to herniate through the incisura of the *tentorium* compressing the midbrain. This situation is referred to as *uncal or lateral herniation* and is a medical emergency because the herniating temporal lobe can exert pressure directly on the lateral aspect of the midbrain interfering with its blood supply. These herniation lesions can destroy ARAS pathways in the midbrain resulting in permanent *coma* or *persistent vegetative state*.

5.2. Reticular Formation and Sleep

The reticular formation plays a prominent role in the elaboration of normal sleep cycle stages through its circuitry with the cortex and diencephalon. Lesions or stimulations of certain regions of the hypothalamus and frontal lobes also affect the sleep cycles.

It is not well understood how the sleep cycle is initiated. However, one view suggests that the *suprachiasmatic nucleus* of the anterior hypothalamus

plays a key role. The suprachiasmatic nucleus receives information regarding the light/dark *diurnal cycle*. Suprachiasmatic nucleus circuitry with the reticular formation suggests important regulatory influences on the pedunculopontine cholinergic, rostral raphe serotonergic, and locus coeruleus noradrenergic neurons. The interactions of these reticular formation nuclei, and their projections to the forebrain, thalamus, and cortex, are believed to be responsible for the production of the various stages of sleep (see Chapter 27).

5.3. Raphe Nuclei of the Medulla Modulate Pain Transmission

Axons of the ascending spinothalamic and trigeminothalamic tracts convey pain sensations from visceral structures and the body and face. These tracts also provide collaterals to the lateral columns of the reticular formation (Fig. 5). This sensory input contributes to the ARAS described in Section 5.1. The serotonergic neurons of raphe and medial column nuclei in the caudal brain stem are also activated by nociceptive input. Specifically, these nuclei include *raphe magnus* and *gigantocellular nuclei* (Fig. 1C, D). The axons of some of these serotonergic neurons distribute to the spinal trigeminal nucleus by means of reticulonuclear tracts. Other serotonergic axons descend in the dorsolateral aspect of the spinal cord white matter and terminate in the dorsal horn (Fig. 4). The serotonin released in the dorsal horn and spinal trigeminal nucleus modulates pain transmission by activating *enkephalinergic interneurons*. Enkephalin inhibits transmission of nociceptive stimuli by pain pathway neurons. Available evidence suggests that a descending *noradrenergic pathway and GABAergic interneurons* are also involved in pain modulation.

Stimulation of the *periaqueductal gray* or the caudal brain stem raphe nuclei induces analgesia. Periaqueductal gray neurons project to the serotonergic neurons of the caudal brain stem probably involving opiate and nonopiate mechanisms (Fig. 5). In certain patients with intractable pain, brief activation of electrodes implanted in the dorsal columns of the spinal cord or in the periaqueductal gray can provide an analgesic effect that lasts for hours or longer.

Recent evidence, acquired with fMRI, demonstrates pain modulation in humans. These data show that the periaqueductal gray is activated by voluntary attentional distraction while subjects are experiencing a painful stimulus. These findings infer that the activation of periaqueductal gray is a key early step in pain modulation in humans. (see Chapter 22 for details.)

5.4. Regulation of Skeletal Muscle Tone, Reflexes, and Body Posture

The reticular formation influences motor activities through its reciprocal connections with red nucleus, substantia nigra, subthalamus, basal ganglia, motor cortex, cerebellum, and spinal cord (Fig. 4 and Fig. 5). Midbrain reticular nuclei and the lateral reticular nucleus of the medulla project to the *inferior olfactory nucleus*, as does the *red nucleus*. The inferior olfactory nucleus sends massive projections to the contralateral cerebellum as the *olivocerebellar tract*. Through these circuits, the reticular formation participates, with other motor and vestibular system components, in providing a continuously integrated regulation of body posture and muscle tone in support of voluntary motor actions.

Reticular formation regulates alpha and gamma lower motor neuron activities via reticulospinal and reticulonuclear tracts that take origin primarily from the medial columns of the reticular formation (Fig. 4). Specifically, the *pontine reticular nucleus* and the *gigantocellular nucleus* of the medulla provide predominately ipsilateral and contralateral projections to spinal cord via the *medial reticulospinal and lateral reticulospinal tracts*, respectively (Fig. 1A, C). In addition, the pedunculopontine nucleus in the pons and midbrain contributes projections to the lower motor neurons. Pontine reticular nucleus projections exert facilitatory effects on neurons innervating axial and limb extensor muscles, whereas input from gigantocellular nucleus inhibits lower motor neurons innervating axial extensors but facilitates motor neurons that innervate limb flexors (Fig. 4).

5.5. Integration of Conjugate Eye Movements

A portion of the medial columns of the reticular formation in the pons is termed the *paramedian pontine reticular formation*, or PPRF (see Fig. 6 in Chapter 12). The PPRF overlaps the pontine reticular nucleus and integrates horizontal eye movements (Fig. 1C). PPRF receives inputs from the superior colliculus, ipsilateral vestibular nuclei, reticular formation, and the contralateral frontal eye fields of the cerebral cortex. It projects primarily to the ipsilateral *abducens nucleus* and, by following the *medial longitudinal fasciculus*, to the portion of the contralateral *oculomotor nucleus* that innervates the contralateral medial rectus muscle. Through these circuits, the PPRF integrates *horizontal conjugate eye movements* in response to head and body position.

A group of neurons that regulate conjugate eye movements in the vertical plane has been located in

the rostral midbrain. Specifically, the *interstitial nucleus of Cajal* and the *rostral interstitial nucleus of the medial longitudinal fasciculus*, and probably other nearby nuclei located in the ventrolateral periaqueductal gray, regulate *conjugate vertical gaze*.

5.6. Regulation of Vital Visceral Functions

Reticular formation neurons that participate in regulation of cardiovascular, respiratory, and other visceral functions are intermingled with reticular neurons and pathways serving other functions described earlier. Terms such as *inspiratory center* refer to observations of particular physiologic responses after stimulation of a region of the reticular formation rather than an anatomically defined cluster of neurons serving only inspiration. Nonetheless, certain areas of the caudal brain stem reticular formation have been shown to influence particular visceral functions.

Anatomic evidence shows that visceral sensory input from most organ systems follows the autonomic nerves to the spinal cord dorsal horn and to the *solitary nucleus* of the brain stem (Fig. 1B). Solitary neurons project to the reticular nuclei of the medial and lateral (parvocellular) columns (Fig. 5). Ascending pathways convey the visceral sensory modalities, including visceral pain, to the reticular formation. Here, sensory information is processed for visceral reflex functions and ascends to the thalamus and hypothalamus. The hypothalamus is the highest center for regulation and integration of visceral functions; it projects this integration to the reticular formation and directly to autonomic centers in the brain stem and spinal cord (Fig. 4) (see Section 3).

As noted above, visceral integration and regulation occur at the nonconscious level with the participation of the hypothalamus, reticular formation, and the autonomic nuclei and nerves. Therefore, the current notion is that higher centers in the limbic system and cerebral cortex can significantly influence visceral regulation through their connections with the hypothalamus and the reticular formation.

Sections 5.6.1 and 5.6.2 describe some aspects of the innervations of the cardiovascular and respiratory systems as examples of the key roles played by the reticular formation in regulating visceral functions.

5.6.1. REGULATION OF CARDIOVASCULAR FUNCTIONS

The carotid sinus and body, located in the bifurcation of the common carotid artery in the neck, are *baroreceptor* (blood pressure sensor) and *chemoreceptor*

(oxygen–carbon dioxide and blood pH sensor) sensory organs, respectively. Similar structures are found in the arch of the aorta. Afferent fibers, following the *glossopharyngeal and vagus nerves*, convey information to the *solitary nucleus*, which is a principal integrator of cardiovascular and respiratory functions (Fig. 1B). Increasing carbon dioxide, hydrogen ion concentrations, changes in blood pressure, and diminishing oxygen concentrations in the blood are potent activators of the solitary nucleus. The solitary neurons form complex circuits with the cardiovascular and respiratory regulatory nuclei primarily in the parvocellular (lateral column) regions of the medullary and caudal pontine reticular formation and with the dorsal motor nucleus and nucleus ambiguus (Fig. 1B, C and Fig. 5).

Two subnuclei of the *ventral lateral reticular nucleus*, located in the parvocellular (lateral column) region of the medulla, are key structures associated with cardiovascular regulation (Fig. 1A, B). Here, the *rostral ventral lateral reticular subnucleus (RVL)* has a *cardiac pressor function*, whereas the *caudal ventral lateral reticular subnucleus (CVL)* is associated with a *cardiac depressor function*. RVL subnucleus has monosynaptic, glutamate-dependent connections with, and provides a tonic activation of, the sympathetic preganglionic neurons in the *intermediolateral nucleus (lateral horn)* in the thoracic spinal cord (Fig. 4). The cholinergic sympathetic preganglionic neurons innervate the postganglionic neurons in the sympathetic chain ganglia; these noradrenergic postganglionic neurons provide sympathetic innervation to the heart and vasculature. The outcome is an increase in heart rate and force and a facilitation of vasoconstriction in most vascular beds. Another outcome is an increase in pulmonary blood flow resulting in an increase in blood oxygen concentration.

Parasympathetic preganglionic neurons that innervate the heart are located in the *dorsal motor nucleus of the vagus* and *nucleus ambiguus* in the medulla. Baroreceptor signals, representing increasing blood pressure, activate two solitary nucleus pathways to reduce the blood pressure. First, the solitary nucleus activates the CVL subnucleus, the depressor portion of ventral lateral reticular nucleus. CVL subnucleus *inhibits* RVL subnucleus, thereby *reducing* the tonic activation of the sympathetic innervation to the heart; the outcome is a reduction in blood pressure. Second, the solitary nucleus also activates the cholinergic parasympathetic preganglionic neurons in the dorsal motor and ambiguus nuclei. The axons of these parasympathetic preganglionic neurons follow

the vagus nerve and terminate on cholinergic parasympathetic postganglionic terminal ganglia near the heart. The outcome of the parasympathetic innervation is a reduction in blood pressure.

Additional regulatory circuits influencing cardiovascular functions involve connections with the *adrenal medulla* and the *area postrema*. The RVL subnucleus projects to those sympathetic preganglionic neurons in the intermediolateral nucleus of the thoracic spinal cord that activate the cells of the *adrenal medulla* resulting in secretion of *epinephrine (adrenaline)* into the blood. Epinephrine increases heart rate and force and facilitates vasoconstriction, thereby increasing blood pressure and blood perfusion in the peripheral tissues. It is important to recognize that the medullary reticular formation has two routes to activate cardiovascular function: first, a quick-acting neuroanatomic pathway through the sympathetic innervation; second, a long-acting, neural-hormonal pathway through the release of epinephrine from the adrenal medulla.

The *area postrema* is a paired *circumventricular organ* located in the medulla just anterior to the caudal aspect of the fourth ventricle. Area postrema neurons are directly exposed to concentrations of substances in the blood and tissue fluids because the area postrema lacks a blood-brain barrier. Area postrema neurons project *glutamate* terminals to the medullary reticular formation and to the solitary nucleus. Available evidence suggests that this blood-neural route can influence cardiovascular and respiratory regulatory neurons in the reticular formation.

5.6.2. REGULATION OF PULMONARY FUNCTION

Inputs from peripheral chemoreceptors, lung stretch receptors, and other afferent pulmonary sources are received by the solitary nucleus and distributed to medullary and pontine reticular formation centers that regulate respiratory function (Fig. 1B and Fig. 5). Reticular formation neurons influencing *respiratory rhythms* are located near the solitary nucleus and nucleus ambiguus in the parvocellular region of the medullary and pontine tegmentum. A *dorsal respiratory subnucleus* is located near, and overlapping, the solitary nucleus; its neurons have an inherent firing rhythm and project to the *phrenic nucleus* at the fourth cervical spinal cord level. The paired *phrenic nerves* innervate the skeletal muscle of the *diaphragm*. Stimulation of the dorsal respiratory subnucleus produces a *ramp signal* (an increasing rate of neural impulses with time) to the diaphragm necessary for inspiratory action. A *ventral respiratory*

subnucleus has been located near the nucleus ambiguus; it is relatively inactive during quiet breathing but becomes active in response to increased demands on inspiration and the forced expiration required during exercise.

A *pneumotaxic center* is located in the rostral pons near the parabrachial nucleus. It regulates breathing rhythms, especially inspiration, via its connections to the dorsal respiratory subnucleus. It is responsible for limiting, or turning off, the ramp signal for inspiration thereby allowing passive exhalation to ensue.

In some newborn infants, the immaturity of these respiratory circuits may alter neural control of respiratory rhythms placing the child at risk for *sudden infant death syndrome (SIDS)*.

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Harold H. Traurig

CONTENTS

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1. INTRODUCTION

The reticular formation is an aggregation of several subtypes of interconnected neurons extending throughout the brain stem tegmentum. It is continuous and functionally related to the hypothalamus and midline and interlaminar nuclei of the dorsal thalamus. The reticular pathways integrate sensory, visceral, limbic, and motor functions. Reticular circuits project throughout the central nervous system and exert important influences on autonomic regulation of vital organ systems, behavior, somatic motor activities, sleep cycles, alertness, and pain modulation.

The term *reticular formation* was adopted by early anatomists to distinguish what appeared to them as diffusely interconnected neurons in the brain stem tegmentum compared with the anatomically more distinct nuclei associated with the cranial nerves. This characterization of the reticular formation suggested that it was poorly organized and served only primitive functions. However, some early investigators, notably Cajal, recognized organization and specificity in the reticular formation based on neuron morphology, location in the brain stem, and connectivity. Despite Cajal's work, the reticular formation evoked little research interest until the electrophysiologic studies by Moruzzi and Magoun in the 1940s and 1950s demonstrated that the maintenance of

consciousness and alertness depended on input from sensory pathways traversing the brain stem reticular formation to the thalamus and cerebral cortex.

Later studies demonstrate that inputs to specific components of the reticular formation originate from cerebral cortex, striatum, limbic structures, hypothalamus, cerebellum, and the spinal cord. Certain components of the reticular formation project to these same structures, often through the thalamus or hypothalamus, as well as to the somatic and visceral motor nuclei of the brain stem and spinal cord.

Additionally, it is evident from numerous recent research reports that subgroups of reticular neurons and nerve terminals contain specific combinations of transmitters and peptides, such as serotonin, norepinephrine, acetylcholine, dopamine, glutamate, gamma-aminobutyric acid (GABA), and enkephalin, suggesting that reticular formation functions also depend on complex neurochemical interactions.

Current evidence reveals that the reticular formation provides an important matrix for neural integration. Ascending reticular formation projections play key roles in alertness, behavior, and affect. Brain stem reticular formation circuits, in concert with limbic and hypothalamic inputs, regulate cardiovascular and respiratory rhythms and other visceral responses through influences on cranial nerve nuclei and descending connections with autonomic centers in the spinal cord. Reticulospinal tracts influence transmission of sensory modalities by dorsal horn neurons and activities of

alpha and gamma motor neurons thereby modulating pain transmission, skeletal muscle tone, and somatic reflexes.

2. ANATOMIC CHARACTERISTICS OF RETICULAR FORMATION NEURONS

The reticular formation is subdivided into a number of nuclei based on their anatomic locations. The more prominent of the reticular nuclei are described here. It is convenient to characterize the reticular formation as three columns of neurons extending throughout most of the brain stem tegmentum (Fig. 1). Although these columns of neurons are not separated by clearly defined anatomic boundaries and often overlap one another, they are distinct from one another based on neuron morphology, neurochemical phenotype, circuitry, and location in the mediolateral plane. The unpaired median column or *raphe nuclei* are located along the midline of the brain stem tegmentum (Fig. 1; *see also* Fig. 3). (Raphe means “seam” and refers to the midline of the brain stem.) The paired medial (central) and lateral columns of nuclei are found in the central (immediately lateral to the midline) and lateral portions of the tegmentum, respectively (Fig. 1). The median and medial columns are the *magnocellular regions*, whereas the lateral column is the *parvocellular region*; these terms reflect the large and small size of the majority of the neurons in these columns, respectively.

Reticular formation neurons possess an aggregate of characteristics that distinguish them from most other brain stem neurons. Many have extensive dendritic arborizations oriented in a plane perpendicular to the long axis of the brain stem and to the ascending sensory and descending motor tracts. Other brain stem neurons have restricted dendritic fields. Reticular formation neuron dendrites typically intermingle with the axons of motor and sensory tracts suggesting functional interactions. Axons of many reticular formation neurons are long, form numerous collaterals, and have ascending and descending branches. Therefore, a single reticular neuron could influence brain stem and spinal cord neurons associated with several different somatic or visceral functions. Inputs to reticular neurons typically originate from many and varied sources. Taken together, these anatomic arrangements reflect the modulatory and integrative roles of the reticular formation.

2.1. Raphe (Median Column) of the Reticular Formation

Several subgroups of ascending and decussating axons traverse the raphe of the brain stem; the more prominent of these are the medial lemniscus, olivocerebellar tract, olivocerebellar tract, and trapezoid body (fibers). Raphe neurons are scattered among these traversing axons most prominently in the medulla and pons. Raphe neurons also extend into the midbrain (squares in Fig. 1; Fig. 2). The following raphe nuclei have been described based on neuron morphology: *raphe obscurus*, *raphe pallidus*, and *raphe magnus* in the medulla (Fig. 1A, B); *raphe magnus*, *raphe pontis*, and *superior central* in the pons (Fig. 1C, D); *dorsal tegmental nucleus* and *nucleus linearis* (Fig. 1E, F) in the midbrain. Many of the raphe neurons contain *serotonin (5-hydroxytryptamine)* and use this *indoleamine* as a transmitter in chemical transmission (Fig. 2).

Raphe serotonergic neurons of the caudal brain stem, such as *raphe magnus*, *raphe pallidus*, and *raphe obscurus*, project prominently to the spinal cord (*see* Fig. 4). Available evidence implies that serotonergic terminals ending in substantia gelatinosa of the dorsal horn play a key role in modulating pain transmission to conscious centers.

Many pontine and midbrain serotonergic raphe neurons, such as *raphe pontis*, *superior central*, and *dorsal tegmental nucleus*, have direct and indirect rostral projections through the diencephalon to amygdala, forebrain, and cerebral cortex. They also project to the noradrenergic neurons of the *locus coeruleus* (Fig. 3 and Fig. 4). These ascending circuits participate in the regulation of alertness and sleep cycles.

2.2. Medial “Effector” Columns of the Reticular Formation

The bilateral medial (central) columns of the reticular formation occupy the central portion of the medullary and pontine tegmentum just lateral to the raphe nuclei (diamonds in Fig. 1). The medial columns are subdivided into the *central reticular* (Fig. 1A, B), *gigantocellular nuclei* in the medulla, *caudal and rostral (oral) pontine reticular nuclei* in the pons (Fig. 1C, D), and rostral pontine reticular nucleus in the midbrain (Fig. 1E). Most of these neurons are large (magnocellular) and have extensive dendritic arborizations oriented perpendicular to the long axis of the brain stem. Long ascending and descending axons, with numerous collateral branches, originate from medial column neurons. These terminate in other regions of the reticular

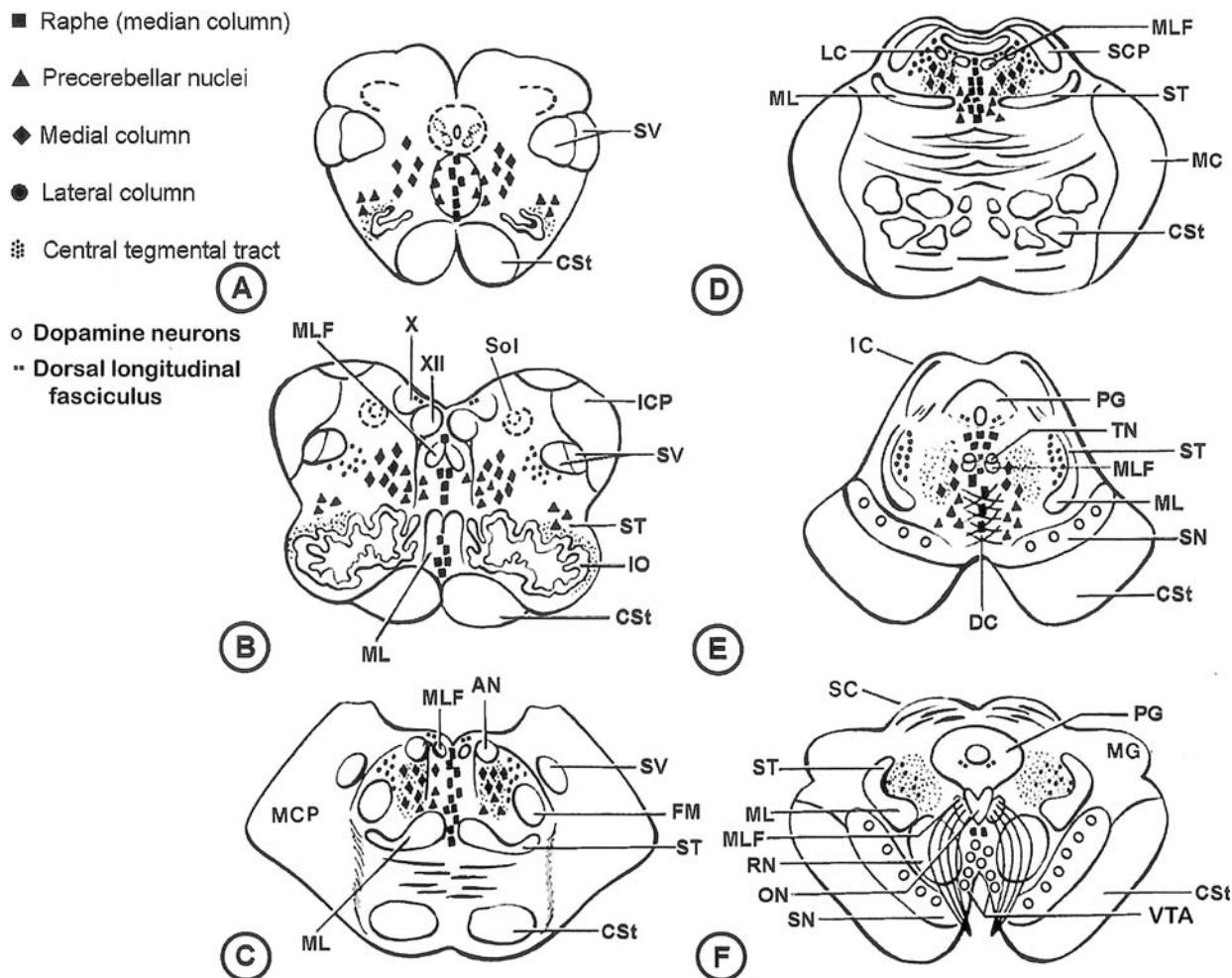


Fig. 1. Representations of the major nuclei of the reticular formation in the brain stem. Anatomic boundaries of these nuclei are indistinct. Numerous subnuclei have been identified based on neuron morphology and neurochemical characteristics. **(A)** Caudal medulla: level of the sensory decussation (medial lemniscus). **(B)** Mid-medulla: level of the vagal and hypoglossal nuclei. **(C)** Caudal pons: level of the facial and abducens nuclei. **(D)** Rostral pons: level of the locus coeruleus. **(E)** Midbrain: level of the inferior colliculus. **(F)** Midbrain: level of the superior colliculus. *Median column or raphe of reticular nuclei* (squares) includes nucleus raphe obscurus and nucleus raphe pallidus (medulla, **A** and **B**), nucleus raphe magnus, nucleus raphe pontis, and superior central nucleus (pons, **C** and **D**), and nucleus linearis and dorsal tegmental nucleus (midbrain, **E** and **F**). *Medial (central) column of reticular nuclei* (diamonds) includes central reticular nucleus and gigantocellular nucleus (medulla, **A** and **B**), caudal and rostral pontine reticular nuclei (pons, **C** and **D**), and rostral pontine reticular nucleus (midbrain, **E**). *Lateral column of reticular nuclei* (large dots) includes ventrolateral reticular nucleus in the parvocellular region (medulla and pons, **B** and **C**), parabrachial nucleus (pons **D** and **E**), and pedunculopontine nucleus and cuneiform nucleus (midbrain, **E** and **F**). *Precerebellar nuclei* (triangles) include paramedian reticular nuclei (medulla and pons, **A**, **B**, and **C**), lateral reticular nucleus (medulla, **A** and **B**), and pontine reticulotegmental nuclei (**D** and **E**). Stippling represents the ascending and descending fibers of the central tegmental tract. Open circles indicate the dopaminergic neurons of the substantia nigra (SN) and the ventral tegmental (mesolimbic) region (VTR). Small squares locate the dorsal longitudinal fasciculus ventral to the cerebral aqueduct and fourth ventricle. Other relevant structures: AN, abducens nucleus; CSt, corticospinal tract; DC, decussation of superior cerebellar peduncle; FM, facial motor nucleus; IC, inferior colliculus; ICP, inferior cerebellar peduncle; IO, inferior olive nucleus; LC, locus coeruleus; MCP, middle cerebellar peduncle; MG, medial geniculate nucleus; ML, medial lemniscus; MLF, medial longitudinal fasciculus; ON, oculomotor nucleus; PG, periaqueductal gray; RN, red nucleus; SC, superior colliculus; SCP, superior cerebellar peduncle; Sol, solitary nucleus; SN, substantia nigra; ST, spinothalamic (anterolateral) tracts; SV, spinal nucleus and tract of the trigeminal; TN, trochlear nucleus; X, dorsal motor nucleus of the vagus; XII, hypoglossal nucleus.

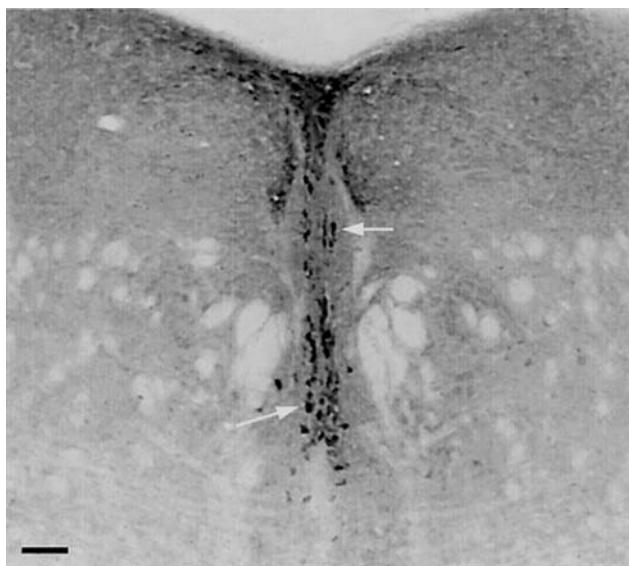


Fig. 2. Serotonergic neurons of the raphe. Immunocytochemical localization of a marker for serotonin in the cell bodies of neurons (arrows) in the brain stem raphe of the mouse. Bar = 100 μ m. (Illustration courtesy of Dr. Melissa Zwick, Richard Stockton College.)

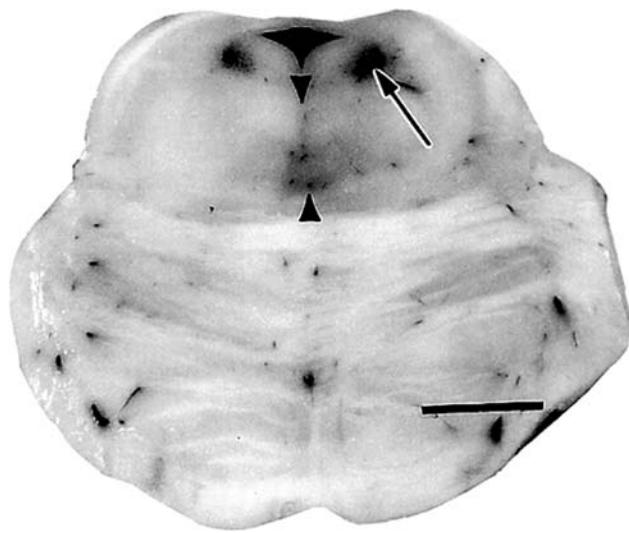


Fig. 3. Locus coeruleus, raphe and central tegmental tract. Unstained section of human rostral pons demonstrating the pigmented, noradrenergic neurons of the locus coeruleus (arrow). The shaft of the arrow overlies the central tegmental tract. Arrowheads indicate the raphe region of the pons. Bar = 5 mm.

formation, limbic structures, hypothalamus, thalamus (e.g., interlaminar nuclei), cranial nerve nuclei, and the spinal cord (Fig. 4 and Fig. 5). The ascending and descending axons of the medial columns, or “effector” neurons, collect as *reticulocortical*, *reticulonuclear*, *reticulohypothalamic*, and *reticulospinal tracts* and convey reticular formation influences throughout the central nervous system.

2.3. Lateral “Afferent” Columns of the Reticular Formation

The principal lateral column nuclei (dots in Fig. 1) are composed mostly of small (parvocellular) neurons and include, the *parvocellular nucleus (region)* and the *ventrolateral nucleus* in the lateral tegmentum of the medulla and pons (Fig. 1B, C), *parabrachial nucleus* in the pons (Fig. 1D), and *pedunculopontine* and *cuneiform nuclei* in the midbrain (Fig. 1F). Lateral column (parvocellular) components in the medulla are located between the medial reticular column medially and the spinal trigeminal nucleus laterally. Rostrally, the lateral column is ventromedial to the superior cerebellar peduncle in the pons and medial to the medial lemniscus and anterolateral tracts in the midbrain.

The parvocellular neurons of the lateral columns are characterized by prominent afferent projections from axon collaterals of most sensory tracts. The sensory projections received by the lateral column neurons are relayed medially to the medial column; the medial column nuclei have reciprocal connections with the raphe nuclei (Fig. 5). For example, the parvocellular neurons in the lateral column in the medulla and pons relay somatic and visceral sensory information from the ascending anterolateral, spinoreticular, and trigeminothalamic tracts to the medial column. Other visceral afferents are conveyed by the facial, glossopharyngeal, and vagus nerves to the *solitary nucleus* in the medulla (Fig. 1B). Solitary neurons relay integrated visceral sensory information to medial and lateral (parvocellular) columns of the reticular formation. Finally, cerebellar, auditory, visual, and vestibular afferents also terminate in the medial and lateral (parvocellular) columns by direct and indirect pathways (Fig. 5).

Functionally, the ascending and descending reticular tracts provide regulatory circuits that modulate many important functions and reflexes (Fig. 4 and Fig. 5). For example, many of these terminals modulate nociceptive transmissions by activating serotonergic raphe nuclei, which, in turn, activate enkephalinergic interneurons in the substantia gelatinosa of the dorsal horn and in the spinal trigeminal nucleus (see Section 5.3 for details). They also activate ascending pathways that follow the *central tegmental tract* and relay in the thalamus. Related thalamocortical fibers project throughout the cerebral cortex to facilitate alertness (Fig. 4; see Section 5.1). Connections of the parabrachial nucleus in the pons and midbrain suggest roles in visceral and limbic functions. The *pedunculopontine* and *cuneiform nuclei* are involved in motor functions based on their projections

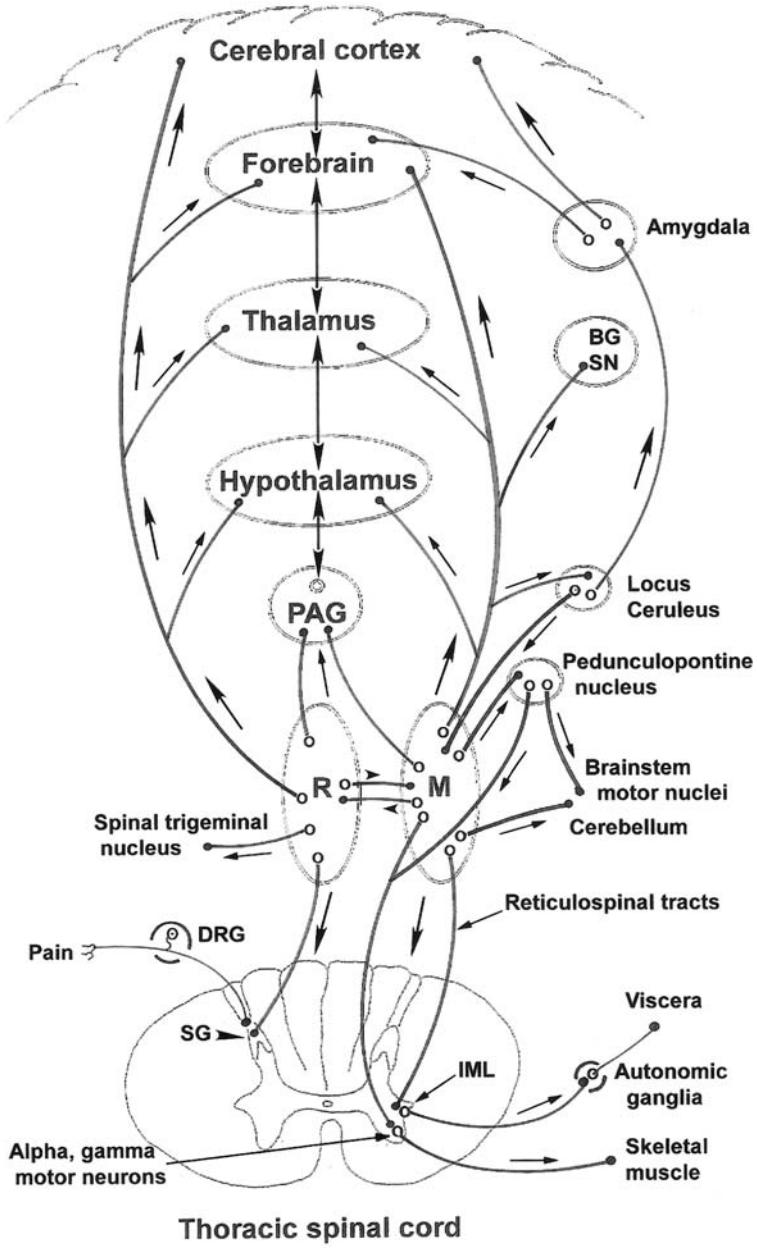


Fig. 4. Bold arrows and arrowheads represent the principal efferent projections of the reticular formation. Neuron cell bodies and terminals are represented by open circles and black dots, respectively. Reticular efferent projections to rostral targets follow the medial forebrain bundle, mamillotegmental tract, dorsal longitudinal fasciculus, central tegmental tract, and other pathways. R, raphe (median column) neurons; M, medial (central) column neurons. Lateral column neurons are represented by the pedunculopontine nucleus. BG, basal ganglia; DRG, dorsal root ganglion; IML, intermediolateral nucleus; PAG, periaqueductal gray; SG, substantia gelatinosa of the dorsal horn; SN, substantia nigra.

to the motor cortex, basal ganglia, substantia nigra, precerebellar, and raphe nuclei (see Section 5.4).

3. RETICULAR FORMATION PATHWAYS

Ascending and descending pathways in the reticular formation are diffuse and multisynaptic. Most efferent projections originate from the magnocellular

nuclei (Fig. 4). Afferent projections to the reticular formation include those from the spinal cord, solitary nucleus, cerebellum, periaqueductal gray, hypothalamus, thalamus, areas of the cerebral cortex, and directly and indirectly from limbic structures (Fig. 5). A few afferent and efferent tracts are described in this section; others are described with their special functions in the following sections of this chapter.

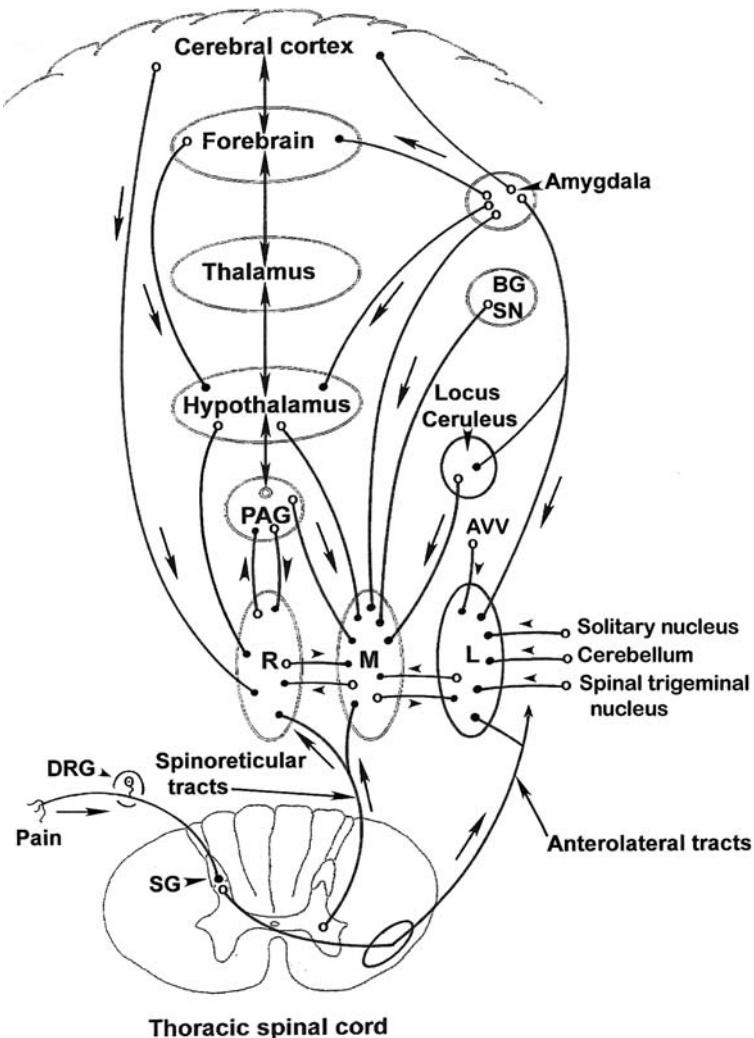


Fig. 5. Bold arrows and arrowheads represent the principal afferent projections to the reticular formation. Neuron cell bodies and terminals are represented by open circles and black dots, respectively. Afferent projections from rostral sources follow medial forebrain bundle, mammillotegmental tract, mammillary peduncle, dorsal longitudinal fasciculus, central tegmental tract, and other pathways to the reticular formation. R, raphe (median column) neurons; M, medial (central) column neurons; L, lateral column (parvocellular) neurons. AVV, afferents from the auditory, vestibular, and visual systems; BG, basal ganglia; DRG, dorsal root ganglia; PAG, periaqueductal gray; SG, substantia gelatinosa of the dorsal horn, SN, substantia nigra.

3.1. Reticulospinal Tracts

The *lateral and medial reticulospinal tracts* arise from the magnocellular neurons of the medial column in the medulla and pons, respectively (Fig. 4). The *medial reticulospinal tract* originates mostly from the ipsilateral *pontine reticular nucleus* and projects to the spinal cord via the ventral funiculus. *Gigantocellular nucleus* in the medulla projects mostly ipsilaterally as the *lateral reticulospinal tract* located in the ventral part of the lateral funiculus of the spinal cord. Both of these projections provide complex regulation of *alpha and gamma motor neurons*.

Other reticulospinal fibers originate from the *lateral tegmental nucleus* located between the solitary and the spinal trigeminal nuclei in the medullary tegmentum. These *cholinergic neurons* project to the *intermediolateral nucleus* in the lateral horn of the thoracic and upper lumbar spinal cord and innervate *sympathetic preganglionic neurons* (Fig. 4). In addition, some reticulospinal fibers terminate on *parasympathetic preganglionic nuclei* and somatic motor neurons in the sacral spinal cord (S_2, S_3, S_4). These projections provide critical links between visceral regulatory centers in the hypothalamus, reticular formation, and autonomic neurons located in the brain stem and spinal cord.

3.2. Spinoreticular Tracts

Visceral sensory fibers return to the central nervous system from most viscera and the vasculature. These modalities include visceral pain and sensory information related to visceral reflexes (e.g., gag reflex) and functions (e.g., micturition) (Fig. 5). The primary sensory fibers follow the sympathetic and parasympathetic nerves and have their cell bodies in dorsal root ganglia or in the sensory ganglia associated with the facial, glossopharyngeal, and vagus nerves.

In the spinal cord, the visceral sensory fibers terminate in the dorsal horn, in the brain stem, most terminate in the solitary nucleus (Fig. 1B). Secondary neurons provide reflex connections with autonomic neurons in the spinal cord and brain stem. In the spinal cord, other secondary fibers form bilateral *spinoreticular tracts* that convey visceral sensations to the reticular formation and indirectly to the periventricular gray, hypothalamus, and thalamus. Some crossed secondary fibers join the anterolateral system and convey visceral sensations to the thalamus. Thalamocortical fibers project to the post central gyrus and the cortex of the insula where visceral pain and other visceral sensations reach consciousness.

3.3. Central Tegmental Tract

The *central tegmental tract* consists of afferent projections to the reticular nuclei and ascending and descending connections between reticular nuclei and other functionally related structures. It extends from midbrain levels to the caudal medulla and is most distinct at caudal midbrain and rostral pontine levels (Fig. 1A–F and Fig. 3). Ascending central tegmental projections arise from *noradrenergic neurons* of the *locus coeruleus* and from *cholinergic neurons* of the *pedunculopontine nucleus* and project to the interlaminar thalamic nucleus, forebrain, and cerebral cortex (Fig. 4). These projections play a key role in maintaining consciousness and in regulating the sleep-wake cycle. Most descending fibers terminate in the *inferior olfactory nucleus* in the medulla and participate in regulation of motor activities (Fig. 1A, B).

3.4. Mammillotegmental Tract and Mammillary Peduncle

Neurons in posterior regions of the hypothalamus, including the mammillary bodies, form the *mammillary fasciculus*. The descending branch of

the mammillary fasciculus projects to the brain stem as the *mammillotegmental tract*. It terminates in the periaqueductal gray and among the nuclei of the midbrain reticular formation. Therefore, the mammillotegmental tract forms an indirect link between the visceral regulatory centers in the posterior hypothalamus through the reticular formation to visceral reflex centers and autonomic nuclei in the brain stem and spinal cord. The *mammillary peduncle* consists mostly of ascending fibers (reticulohypothalamic tract) from the midbrain reticular formation to the posterior hypothalamus (Fig. 4 and Fig. 5).

3.5. Dorsal Longitudinal Fasciculus

Neurons in the periventricular and other regions of the hypothalamus project to the periaqueductal gray and the rostral reticular formation as the *dorsal longitudinal fasciculus*. It is a diffuse collection of small fibers located in the periaqueductal gray just anterior to the aqueduct and fourth ventricle (small squares in Fig. 1B–F). As described for the mammillotegmental tract, the dorsal longitudinal fasciculus provides an indirect link, through the reticular formation, between the hypothalamus and visceral reflex and autonomic centers in the brain stem and spinal cord.

3.6. Medial Forebrain Bundle

The *medial forebrain bundle* contains ascending and descending fibers that course through the lateral hypothalamus making synaptic contact with various hypothalamic nuclei. It connects limbic forebrain structures and lateral hypothalamic nuclei with the rostral reticular formation and periaqueductal gray. The *amygdala*, particularly its central nucleus, projects via the medial forebrain bundle to the midbrain reticular formation (Fig. 4).

3.7. Direct Pathways to Brain Stem and Spinal Cord Autonomic Nuclei

The hypothalamus projects *direct hypothalamomedullary and hypothalamospinal* fibers to the brain stem and spinal cord autonomic nuclei. Many of these descending fibers follow the reticular formation pathways described above and traverse the brain stem reticular formation to reach their targets. The hypothalamospinal fibers course through the lateral funiculus of the spinal cord to reach their targets, the preganglionic sympathetic neurons in the

intermediolateral nucleus in the lateral horn at thoracic and lumbar levels, and the preganglionic parasympathetic nuclei at sacral levels.

4. OTHERS RELATED BRAIN STEM NUCLEI

Other named brain stem nuclei are functionally closely related to the reticular formation. They are briefly described below and considered in detail in other chapters.

4.1. Substantia Nigra and Basal Ganglia

The *substantia nigra* of the midbrain consists of *compact and reticular regions*. Neurons of the compact portion express the catecholamine neurotransmitter *dopamine* (Fig. 1E, F). The dopaminergic compact region neurons project to the striatum (putamen and caudate nucleus). In the striatum, dopamine has both facilitatory and inhibitory effects depending on the receptor type expressed by striatal neurons. The dopaminergic projection to the striatum plays a key role in regulating the posture and muscle tone associated with voluntary movements. Degeneration of the dopaminergic substantia nigra neurons results in *Parkinson's disease*.

The substantia nigra reticular region receives *GABAergic* and *substance P* projections from the striatum and provides GABAergic innervation to the substantia nigra dopaminergic neurons, the reticular formation, and ventral anterior and ventral lateral nuclei of the thalamus. The basal ganglia and the substantia nigra have reciprocal connections with the midbrain and rostral pontine reticular formation, specifically with the serotonergic rostral raphe nuclei and the cholinergic pedunculopontine nucleus (Fig. 4 and Fig. 5). These circuits provide important functional links between the motor regulatory centers in the motor cortex and basal ganglia and the reticular formation. Reticulonuclear and reticulospinal tracts project reticular formation regulation to the alpha and gamma motor neurons associated with the skeletal muscles innervated by the cranial and spinal nerves.

4.2. Red Nucleus

The *red nucleus* occupies the central midbrain tegmentum (Fig. 1F). Its functional role is exclusively related to contralateral motor regulation. The red nucleus receives inputs predominately from the ipsilateral motor cortex, spinal cord, and contralateral cerebellum. In addition, it is reciprocally connected with the lateral reticular nucleus in the medulla, which also projects to the cerebellum. It gives rise to

contralateral *rubronuclear* and *rubrospinal tracts* that terminate on brain stem reticular neurons, cranial nerve motor nuclei, and the motor neurons of the rostral portion of the spinal cord. Another major projection of the red nucleus follows the *central tegmental tract* (stippling in Fig. 1) to the ipsilateral *inferior olfactory nucleus* (Fig. 1A, B).

4.3. Precerebellar Nuclei

Three reticular formation nuclei in the caudal brain stem, collectively termed the *precerebellar reticular nuclei*, have restricted functional roles related to the motor system because they project almost exclusively to the cerebellum (triangles in Fig. 1). Two of these nuclei, the *paramedian reticular* (Fig. 1A–C) and the *pontine reticulotegmental nuclei* (Fig. 1D, E), form a parallel column of neurons located between the medial column of the reticular formation and the brain stem raphe. The third precerebellar reticular nucleus, the *lateral reticular nucleus* (Fig. 1A, B), is a conspicuous cluster of large neurons in the lateral tegmentum of the medulla lying between the spinal trigeminal system and the inferior olfactory nucleus. Together, these precerebellar nuclei provide integrating circuits between cerebellum, red nucleus, inferior olfactory nucleus, and reticular formation that participate in regulating motor functions such as posture, equilibrium, and muscle tone.

4.4. Inferior Olivary Nucleus

The *inferior olivary nucleus* (or complex) is a prominent structure in the ventral tegmentum of the medulla. It includes two smaller components, the dorsal and medial accessory olfactory nuclei. The inferior olivary nucleus has connections similar to those of the precerebellar nuclei of the reticular formation. It receives input from the contralateral spinal cord (spino-olivary tract), indirect input from the reticular formation, and a large direct input from the ipsilateral red nucleus by way of the central tegmental tract (Fig. 1 and Fig. 3). Olivocerebellar fibers cross the midline through the medullary reticular formation and enter the cerebellum via the *inferior cerebellar peduncle*. Virtually all olivocerebellar fibers terminate as climbing fibers on *Purkinje neuron* dendrites in the contralateral cerebellar cortex. The cerebellum projects prominently to the contralateral red nucleus via the *dentatorubrothalamic tract*. Therefore, a cerebellum-red nucleus-inferior olivary nucleus-cerebellum circuit is established that plays a key role in regulation of motor activities.

4.5. Periaqueductal Gray

The *periaqueductal gray* surrounds the cerebral aqueduct and consists of numerous axonal terminals and subgroups of neurons containing many different transmitters and peptides (Fig. 1E, F). Many of these neurons have extensive dendrites that intermingle with the midbrain reticular formation. Periaqueductal gray receives its most prominent inputs from the hypothalamus, forebrain, and limbic structures (Fig. 5). Afferents also originate from the parabrachial and other reticular nuclei, the solitary nucleus, and the ascending sensory pathways. Periaqueductal gray provides reciprocal projections to all of the above structures and to the serotonergic raphe nuclei of the brain stem (Fig. 4). Specifically, periaqueductal gray projects to the nucleus raphe magnus in the pons and medulla (Fig. 1C). Nucleus raphe magnus projects serotonergic terminals to the substantia gelatinosa of the spinal cord and to the trigeminal spinal nucleus where the synaptic release of serotonin activates enkephalergic interneurons. The enkephalergic neurons modulate nociceptive stimuli by diminishing activities in the transmission neurons whose axons form the spinothalamic and trigeminothalamic tracts. Thus, the periaqueductal gray, in concert with the raphe nuclei of the reticular formation, plays a key role in integrating limbic and autonomic functions and in modulating pain perceptions (see Chapter 21).

4.6. Ventral Tegmental Region

The *ventral tegmental region* or *mesolimbic region of Tsai* is located in the posterior perforated substance of the ventromedial aspect of the midbrain (Fig. 1F). It receives serotonergic terminals from the rostral raphe nuclei as well as input from other reticular neurons. The ventral tegmental region receives indirect input from the ascending sensory tracts through the reticular formation. The ventral tegmental neurons are dopaminergic and project to *medio-basal frontal cortex, cingulate gyrus, hippocampus, amygdala, nucleus accumbens, basal nucleus of Meynert*, and other limbic and cortical regions.

Accumulating evidence implies that the *mesolimbic* and *mesocortical* dopaminergic projections from the ventral tegmental region play key roles in *alertness, emotion, and cognition*. Schizophrenic symptoms may result from inappropriate activation of the mesolimbic and mesocortical dopamine projections to the forebrain and other limbic structures. In addition, evidence suggests that drugs of abuse activate the mesolimbic and mesocortical projections to those

limbic structures that generate feelings of pleasure and reward. Because activities of some dopaminergic neurons are facilitated by serotonin, it is likely that serotonergic raphe neurons are also an important component of the circuitry that regulates limbic system functions. Available evidence suggests that the development of *schizophrenia* involves other factors such as multiple dopamine receptors and alterations in responsiveness of mesolimbic and/or mesocortical dopamine targets.

4.7. Locus Coeruleus

The *locus coeruleus* is a compact group of pigmented neurons located in the rostral pons ventrolateral to the fourth ventricle and dorsolateral to the *pontine reticular nucleus* (Fig. 1D and Fig. 3). Locus coeruleus neurons express *norepinephrine*, a catecholamine transmitter. Afferents to locus coeruleus arise from the rostral serotonergic raphe nuclei and the cholinergic pedunculopontine nucleus in the lateral tegmentum of the midbrain (Fig. 4). One subgroup of these afferents exerts inhibitory influences, another facilitates locus coeruleus activity. Other locus coeruleus afferents arise from the cerebral cortex, amygdala, hippocampus, periaqueductal gray, hypothalamus, and from limbic structures especially the forebrain. The major efferents of the locus coeruleus are to these same structures and to other reticular formation nuclei (Fig. 5).

These circuits allow the locus coeruleus to respond to new sensory stimuli and, through its widely disseminated noradrenergic projections, exert an alerting and activating influence throughout the brain. In addition, norepinephrine released from the locus coeruleus plays a role in the sleep-wake cycle.

Depression is thought to involve diminished norepinephrine release by locus coeruleus and other neurons. However, current evidence infers that the neurochemical basis for depression is complex and very likely involves other transmitter systems, such as serotonin.

5. RETICULAR FORMATION FUNCTIONS AND INTERACTIONS

Reticular formation morphology, circuitry, and chemical transmission characteristics provide an important matrix for integration of many neural functions. The control of vital functions, such as regulation of blood pressure, depends on integrated circuitry between cortex, diencephalon, reticular formation, spinal cord, and peripheral tissues. To that end,

inputs converge on the reticular formation from almost all somatic and visceral sensory pathways and from cortex, hypothalamus, striatum, limbic structures, and spinal cord (Fig. 5). Moreover, the reticular formation provides prominent projections to these same structures (Fig. 4). The reticular formation also plays key roles in alertness, sleep, control of posture and muscle tone, and in pain modulation.

5.1. The Alerting Response

Early studies demonstrated that stimulation of the reticular formation evokes changes in cortical activity characteristic of the arousal induced by sensory stimulation. Later studies revealed that the ascending spinothalamic and trigeminothalamic tracts provide collateral input to the lateral columns of the reticular formation, for example, the parvocellular nucleus in the pons (Fig. 1C–E). The lateral column neurons project to the medial columns in the medulla and pons, specifically the central (ventral) reticular, gigantocellular, and pontine reticular nuclei (Fig. 1A–D). The medial columns form prominent ascending projections that follow the *central tegmental tract* and terminate in the adrenergic locus coeruleus, cholinergic pedunculopontine nucleus, hypothalamus, and the intralaminar nuclei of the dorsal thalamus (Fig. 3 and Fig. 4; *see also* stippling in Fig. 1). Related thalamocortical fibers relay activation throughout the limbic system and cerebral cortex. Additionally, activation of the locus coeruleus noradrenergic projections to the cortex facilitates the attentional state. Collectively, these rostral projections constitute the *ascending reticular activating system (ARAS)* that supports several important functions. The flow of sensory stimuli through ARAS activates the hypothalamic and limbic structures that regulate emotional and behavioral responses (e.g., responses to pain). More important, the flow of sensory stimuli facilitates cortical activity. For example, activation of the widely disseminated locus coeruleus noradrenergic projections to the cortex is important in facilitating the attentional state and in generating sleep/wake cycles (Fig. 5). Other examples of ARAS activities include the alerting responses to a sudden loud sound, a flash of light, smelling salts, or a splash of cold water in the face. Without cortical activation by ARAS, the individual is less able to detect new specific stimuli, and the level of consciousness is diminished.

There are important clinical implications related to ARAS function. The reticular formation projections in the ARAS traverse the midbrain tegmentum; some

of these projections follow the central tegmental tract (Fig. 1 and Fig. 3). Lesions of the midbrain can interrupt the ARAS leading to *altered levels of consciousness or coma* due to the diminished facilitation of limbic and cortical neurons. Lesions frequently affecting the midbrain include cerebrovascular accidents (i.e., stroke) and head trauma. Cerebrovascular accidents can interfere with the blood supply to the brain stem and therefore alter consciousness because of diminished oxygen supply to reticular neurons and ascending pathways.

This discussion should not suggest that the reticular formation is the “center for consciousness.” Experimental and clinical observations imply that consciousness, which is a person’s ability to be aware of self and environment and to orient toward new stimuli, results from the integrated actions of a number of neural structures including the reticular formation. In that regard, positron emission tomography (PET) scan and functional magnetic resonance imaging (fMRI) studies in humans reveal that metabolic activities are depressed in the reticular formation of the rostral pons and midbrain, the periaqueductal gray, and the thalamus as alertness and consciousness are lost after administration of anesthetic drugs.

Head trauma can induce increased intracranial pressure due to collection of blood (i.e., hematoma) between the skull and the brain or the accumulation of edema fluid in the injured brain. Because the brain is encased in the skull, the increased intracranial pressure causes the medial aspect of the temporal lobe, specifically the *uncus of the parahippocampal gyrus*, to herniate through the incisura of the *tentorium* compressing the midbrain. This situation is referred to as *uncal or lateral herniation* and is a medical emergency because the herniating temporal lobe can exert pressure directly on the lateral aspect of the midbrain interfering with its blood supply. These herniation lesions can destroy ARAS pathways in the midbrain resulting in permanent *coma* or *persistent vegetative state*.

5.2. Reticular Formation and Sleep

The reticular formation plays a prominent role in the elaboration of normal sleep cycle stages through its circuitry with the cortex and diencephalon. Lesions or stimulations of certain regions of the hypothalamus and frontal lobes also affect the sleep cycles.

It is not well understood how the sleep cycle is initiated. However, one view suggests that the *suprachiasmatic nucleus* of the anterior hypothalamus

plays a key role. The suprachiasmatic nucleus receives information regarding the light/dark *diurnal cycle*. Suprachiasmatic nucleus circuitry with the reticular formation suggests important regulatory influences on the pedunculopontine cholinergic, rostral raphe serotonergic, and locus coeruleus noradrenergic neurons. The interactions of these reticular formation nuclei, and their projections to the forebrain, thalamus, and cortex, are believed to be responsible for the production of the various stages of sleep (see Chapter 27).

5.3. Raphe Nuclei of the Medulla Modulate Pain Transmission

Axons of the ascending spinothalamic and trigeminothalamic tracts convey pain sensations from visceral structures and the body and face. These tracts also provide collaterals to the lateral columns of the reticular formation (Fig. 5). This sensory input contributes to the ARAS described in Section 5.1. The serotonergic neurons of raphe and medial column nuclei in the caudal brain stem are also activated by nociceptive input. Specifically, these nuclei include *raphe magnus* and *gigantocellular nuclei* (Fig. 1C, D). The axons of some of these serotonergic neurons distribute to the spinal trigeminal nucleus by means of reticulonuclear tracts. Other serotonergic axons descend in the dorsolateral aspect of the spinal cord white matter and terminate in the dorsal horn (Fig. 4). The serotonin released in the dorsal horn and spinal trigeminal nucleus modulates pain transmission by activating *enkephalinergic interneurons*. Enkephalin inhibits transmission of nociceptive stimuli by pain pathway neurons. Available evidence suggests that a descending *noradrenergic pathway and GABAergic interneurons* are also involved in pain modulation.

Stimulation of the *periaqueductal gray* or the caudal brain stem raphe nuclei induces analgesia. Periaqueductal gray neurons project to the serotonergic neurons of the caudal brain stem probably involving opiate and nonopiate mechanisms (Fig. 5). In certain patients with intractable pain, brief activation of electrodes implanted in the dorsal columns of the spinal cord or in the periaqueductal gray can provide an analgesic effect that lasts for hours or longer.

Recent evidence, acquired with fMRI, demonstrates pain modulation in humans. These data show that the periaqueductal gray is activated by voluntary attentional distraction while subjects are experiencing a painful stimulus. These findings infer that the activation of periaqueductal gray is a key early step in pain modulation in humans. (see Chapter 22 for details.)

5.4. Regulation of Skeletal Muscle Tone, Reflexes, and Body Posture

The reticular formation influences motor activities through its reciprocal connections with red nucleus, substantia nigra, subthalamus, basal ganglia, motor cortex, cerebellum, and spinal cord (Fig. 4 and Fig. 5). Midbrain reticular nuclei and the lateral reticular nucleus of the medulla project to the *inferior olfactory nucleus*, as does the *red nucleus*. The inferior olfactory nucleus sends massive projections to the contralateral cerebellum as the *olivocerebellar tract*. Through these circuits, the reticular formation participates, with other motor and vestibular system components, in providing a continuously integrated regulation of body posture and muscle tone in support of voluntary motor actions.

Reticular formation regulates alpha and gamma lower motor neuron activities via reticulospinal and reticulonuclear tracts that take origin primarily from the medial columns of the reticular formation (Fig. 4). Specifically, the *pontine reticular nucleus* and the *gigantocellular nucleus* of the medulla provide predominately ipsilateral and contralateral projections to spinal cord via the *medial reticulospinal and lateral reticulospinal tracts*, respectively (Fig. 1A, C). In addition, the pedunculopontine nucleus in the pons and midbrain contributes projections to the lower motor neurons. Pontine reticular nucleus projections exert facilitatory effects on neurons innervating axial and limb extensor muscles, whereas input from gigantocellular nucleus inhibits lower motor neurons innervating axial extensors but facilitates motor neurons that innervate limb flexors (Fig. 4).

5.5. Integration of Conjugate Eye Movements

A portion of the medial columns of the reticular formation in the pons is termed the *paramedian pontine reticular formation*, or PPRF (see Fig. 6 in Chapter 12). The PPRF overlaps the pontine reticular nucleus and integrates horizontal eye movements (Fig. 1C). PPRF receives inputs from the superior colliculus, ipsilateral vestibular nuclei, reticular formation, and the contralateral frontal eye fields of the cerebral cortex. It projects primarily to the ipsilateral *abducens nucleus* and, by following the *medial longitudinal fasciculus*, to the portion of the contralateral *oculomotor nucleus* that innervates the contralateral medial rectus muscle. Through these circuits, the PPRF integrates *horizontal conjugate eye movements* in response to head and body position.

A group of neurons that regulate conjugate eye movements in the vertical plane has been located in

the rostral midbrain. Specifically, the *interstitial nucleus of Cajal* and the *rostral interstitial nucleus of the medial longitudinal fasciculus*, and probably other nearby nuclei located in the ventrolateral periaqueductal gray, regulate *conjugate vertical gaze*.

5.6. Regulation of Vital Visceral Functions

Reticular formation neurons that participate in regulation of cardiovascular, respiratory, and other visceral functions are intermingled with reticular neurons and pathways serving other functions described earlier. Terms such as *inspiratory center* refer to observations of particular physiologic responses after stimulation of a region of the reticular formation rather than an anatomically defined cluster of neurons serving only inspiration. Nonetheless, certain areas of the caudal brain stem reticular formation have been shown to influence particular visceral functions.

Anatomic evidence shows that visceral sensory input from most organ systems follows the autonomic nerves to the spinal cord dorsal horn and to the *solitary nucleus* of the brain stem (Fig. 1B). Solitary neurons project to the reticular nuclei of the medial and lateral (parvocellular) columns (Fig. 5). Ascending pathways convey the visceral sensory modalities, including visceral pain, to the reticular formation. Here, sensory information is processed for visceral reflex functions and ascends to the thalamus and hypothalamus. The hypothalamus is the highest center for regulation and integration of visceral functions; it projects this integration to the reticular formation and directly to autonomic centers in the brain stem and spinal cord (Fig. 4) (see Section 3).

As noted above, visceral integration and regulation occur at the nonconscious level with the participation of the hypothalamus, reticular formation, and the autonomic nuclei and nerves. Therefore, the current notion is that higher centers in the limbic system and cerebral cortex can significantly influence visceral regulation through their connections with the hypothalamus and the reticular formation.

Sections 5.6.1 and 5.6.2 describe some aspects of the innervations of the cardiovascular and respiratory systems as examples of the key roles played by the reticular formation in regulating visceral functions.

5.6.1. REGULATION OF CARDIOVASCULAR FUNCTIONS

The carotid sinus and body, located in the bifurcation of the common carotid artery in the neck, are *baroreceptor* (blood pressure sensor) and *chemoreceptor*

(oxygen–carbon dioxide and blood pH sensor) sensory organs, respectively. Similar structures are found in the arch of the aorta. Afferent fibers, following the *glossopharyngeal and vagus nerves*, convey information to the *solitary nucleus*, which is a principal integrator of cardiovascular and respiratory functions (Fig. 1B). Increasing carbon dioxide, hydrogen ion concentrations, changes in blood pressure, and diminishing oxygen concentrations in the blood are potent activators of the solitary nucleus. The solitary neurons form complex circuits with the cardiovascular and respiratory regulatory nuclei primarily in the parvocellular (lateral column) regions of the medullary and caudal pontine reticular formation and with the dorsal motor nucleus and nucleus ambiguus (Fig. 1B, C and Fig. 5).

Two subnuclei of the *ventral lateral reticular nucleus*, located in the parvocellular (lateral column) region of the medulla, are key structures associated with cardiovascular regulation (Fig. 1A, B). Here, the *rostral ventral lateral reticular subnucleus (RVL)* has a *cardiac pressor function*, whereas the *caudal ventral lateral reticular subnucleus (CVL)* is associated with a *cardiac depressor function*. RVL subnucleus has monosynaptic, glutamate-dependent connections with, and provides a tonic activation of, the sympathetic preganglionic neurons in the *intermediolateral nucleus (lateral horn)* in the thoracic spinal cord (Fig. 4). The cholinergic sympathetic preganglionic neurons innervate the postganglionic neurons in the sympathetic chain ganglia; these noradrenergic postganglionic neurons provide sympathetic innervation to the heart and vasculature. The outcome is an increase in heart rate and force and a facilitation of vasoconstriction in most vascular beds. Another outcome is an increase in pulmonary blood flow resulting in an increase in blood oxygen concentration.

Parasympathetic preganglionic neurons that innervate the heart are located in the *dorsal motor nucleus of the vagus* and *nucleus ambiguus* in the medulla. Baroreceptor signals, representing increasing blood pressure, activate two solitary nucleus pathways to reduce the blood pressure. First, the solitary nucleus activates the CVL subnucleus, the depressor portion of ventral lateral reticular nucleus. CVL subnucleus *inhibits* RVL subnucleus, thereby *reducing* the tonic activation of the sympathetic innervation to the heart; the outcome is a reduction in blood pressure. Second, the solitary nucleus also activates the cholinergic parasympathetic preganglionic neurons in the dorsal motor and ambiguus nuclei. The axons of these parasympathetic preganglionic neurons follow

the vagus nerve and terminate on cholinergic parasympathetic postganglionic terminal ganglia near the heart. The outcome of the parasympathetic innervation is a reduction in blood pressure.

Additional regulatory circuits influencing cardiovascular functions involve connections with the *adrenal medulla* and the *area postrema*. The RVL subnucleus projects to those sympathetic preganglionic neurons in the intermediolateral nucleus of the thoracic spinal cord that activate the cells of the *adrenal medulla* resulting in secretion of *epinephrine (adrenaline)* into the blood. Epinephrine increases heart rate and force and facilitates vasoconstriction, thereby increasing blood pressure and blood perfusion in the peripheral tissues. It is important to recognize that the medullary reticular formation has two routes to activate cardiovascular function: first, a quick-acting neuroanatomic pathway through the sympathetic innervation; second, a long-acting, neural-hormonal pathway through the release of epinephrine from the adrenal medulla.

The *area postrema* is a paired *circumventricular organ* located in the medulla just anterior to the caudal aspect of the fourth ventricle. Area postrema neurons are directly exposed to concentrations of substances in the blood and tissue fluids because the area postrema lacks a blood-brain barrier. Area postrema neurons project *glutamate* terminals to the medullary reticular formation and to the solitary nucleus. Available evidence suggests that this blood-neural route can influence cardiovascular and respiratory regulatory neurons in the reticular formation.

5.6.2. REGULATION OF PULMONARY FUNCTION

Inputs from peripheral chemoreceptors, lung stretch receptors, and other afferent pulmonary sources are received by the solitary nucleus and distributed to medullary and pontine reticular formation centers that regulate respiratory function (Fig. 1B and Fig. 5). Reticular formation neurons influencing *respiratory rhythms* are located near the solitary nucleus and nucleus ambiguus in the parvocellular region of the medullary and pontine tegmentum. A *dorsal respiratory subnucleus* is located near, and overlapping, the solitary nucleus; its neurons have an inherent firing rhythm and project to the *phrenic nucleus* at the fourth cervical spinal cord level. The paired *phrenic nerves* innervate the skeletal muscle of the *diaphragm*. Stimulation of the dorsal respiratory subnucleus produces a *ramp signal* (an increasing rate of neural impulses with time) to the diaphragm necessary for inspiratory action. A *ventral respiratory*

subnucleus has been located near the nucleus ambiguus; it is relatively inactive during quiet breathing but becomes active in response to increased demands on inspiration and the forced expiration required during exercise.

A *pneumotaxic center* is located in the rostral pons near the parabrachial nucleus. It regulates breathing rhythms, especially inspiration, via its connections to the dorsal respiratory subnucleus. It is responsible for limiting, or turning off, the ramp signal for inspiration thereby allowing passive exhalation to ensue.

In some newborn infants, the immaturity of these respiratory circuits may alter neural control of respiratory rhythms placing the child at risk for *sudden infant death syndrome (SIDS)*.

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Harold H Traurig

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1. INTRODUCTION

The trigeminal nerve, cranial nerve V, provides sensory innervation to the face and structures in the oral and nasal cavities; in addition, its motor component innervates the muscles of mastication and other skeletal muscles. Fine (discriminatory) tactile, general (light) tactile, proprioceptive, thermal, and pain sensory modalities are conveyed to the trigeminal nuclei in the brain stem. Axons from the sensory trigeminal nuclei contribute to important reflex circuits and relay sensory modalities to the thalamus for further integration. Thalamocortical projections relay sensations to the face area of the contralateral cerebral hemisphere, specifically the postcentral gyrus.

The trigeminal system is frequently involved in important clinical conditions because its peripheral and central components have extensive anatomic distributions in the face, cranial cavity and brain stem.

2. PERIPHERAL DISTRIBUTION OF THE TRIGEMINAL NERVE

2.1. The Three Divisions of the Trigeminal Nerve

2.1.1. TRIGEMINAL NERVE

The peripheral branches of the trigeminal nerve coalesce to form the three major divisions: *ophthalmic* (V_1), *maxillary* (V_2), and *mandibular* (V_3) nerves (see Fig. 3). The smaller trigeminal motor component accompanies the mandibular nerve. The *ophthalmic* (V_1), *maxillary* (V_2), and *mandibular* (V_3) nerves enter the middle cranial cavity of the skull through the *superior orbital fissure*, *foramen rotundum*, and *foramen ovale*, respectively. The sensory fibers have their cell bodies of origin in the *trigeminal ganglion*, which is located in a dural pocket (Meckel's cave) on the anterior slope of the *petrous portion of the temporal bone* (Fig. 1). The central processes of the sensory fibers, together with the motor fibers, emerge from the trigeminal ganglion as the *trigeminal nerve*. The trigeminal nerve crosses the ridge (medial aspect) of the petrous portion of the temporal bone inferior to the superior petrosal sinus and enters the posterior cranial cavity. As the trigeminal sensory fibers enter the pons, those fibers conveying sensory modalities from the mandibular nerve are located posterolateral, the ophthalmic nerve fibers are anteromedial, and those of the maxillary division are intermediate in position. In the pons, the trigeminal nerve fibers

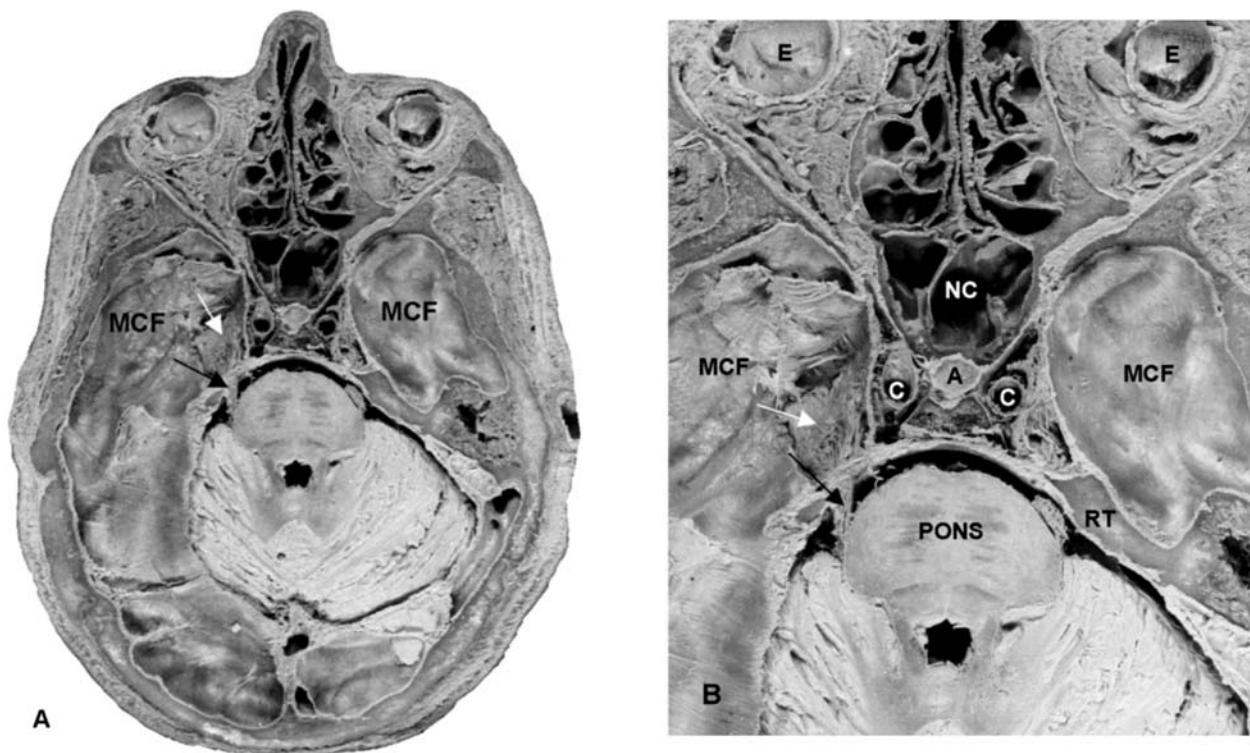


Fig. 1. (A, B) Axial section of the head showing the position of the trigeminal ganglion (white arrow) on the anterior slope of the petrous portion of the temporal bone (RT) in the middle cranial fossa or cavity (MCF). The root of the trigeminal nerve courses posteriorly into the posterior cranial cavity and enters the pons (black arrow). Anterior direction is toward the top of the figure. The plane of the section is inclined superiorly, approximately 1 cm on the left side of the specimen. The section passes through the apex of the tentorium cerebelli and reveals the pons and cerebellum in the posterior cranial fossa. **(B)** Central portion of (A). Trigeminal ganglion is encased in a dural sleeve, which is reflected anteriorly to reveal the ganglion (white arrow). Trigeminal ganglion lies inferior and medial to the temporal lobe, which has been removed in this specimen. In its course, the trigeminal root (black arrow) crosses the petrous ridge of the temporal bone (RT) and passes inferior to the superior petrosal venous sinus. The lateral wall of the cavernous sinus is just medial to trigeminal ganglion and lateral to the internal carotid artery (labeled C). A, adenohypophysis in the sella turcica; C, internal carotid arteries, which are evident just lateral to the sella turcica as they course through the cavernous sinuses; E, eye; NC, nasal cavity.

interdigitate with those of the *middle cerebellar peduncle*; in Fig. 5D, the trigeminal nerve fibers are located between the principle sensory nucleus of the trigeminal (PSnV) and the motor nucleus (MnV).

Near its point of attachment to the pons, the trigeminal nerve is anatomically closely related to arterial branches of the vertebro-basilar system and *superior petrosal vein* (*of Dandy*). Compression or irritation of the trigeminal nerve by these vessels could evoke the pain syndrome known as *trigeminal neuralgia (tic douloureux)*, described later.

2.1.2. THE OPHTHALMIC DIVISION OF THE TRIGEMINAL NERVE

The *ophthalmic nerve* passes forward from the trigeminal ganglion in the middle cranial cavity and becomes embedded in the lateral wall of the *cavernous sinus* (Fig. 1). It continues through the *superior orbital fissure*,

enters the orbit, and divides into its peripheral branches. The *ophthalmic nerve* conveys sensory innervation from the supratentorial dura, globe of the eye, cornea, upper eyelid, forehead regions of the face and scalp, and the mucosae of the ethmoidal, sphenoidal, and frontal sinuses (Fig. 2).

2.1.3. THE MAXILLARY DIVISION OF THE TRIGEMINAL NERVE

The *maxillary nerve* passes forward from the trigeminal ganglion coursing on the floor of the middle cranial cavity and exits through the *foramen rotundum*. Immediately upon exit, the maxillary nerve enters the *pterygopalatine fossa*, which is bounded by the palatine bone medially, the pterygoid process posteriorly, and the maxilla anteriorly. Two other important contents of the pterygopalatine fossa are branches of the *maxillary artery* and the parasympathetic *pterygopalatine ganglion*. Most maxillary nerve

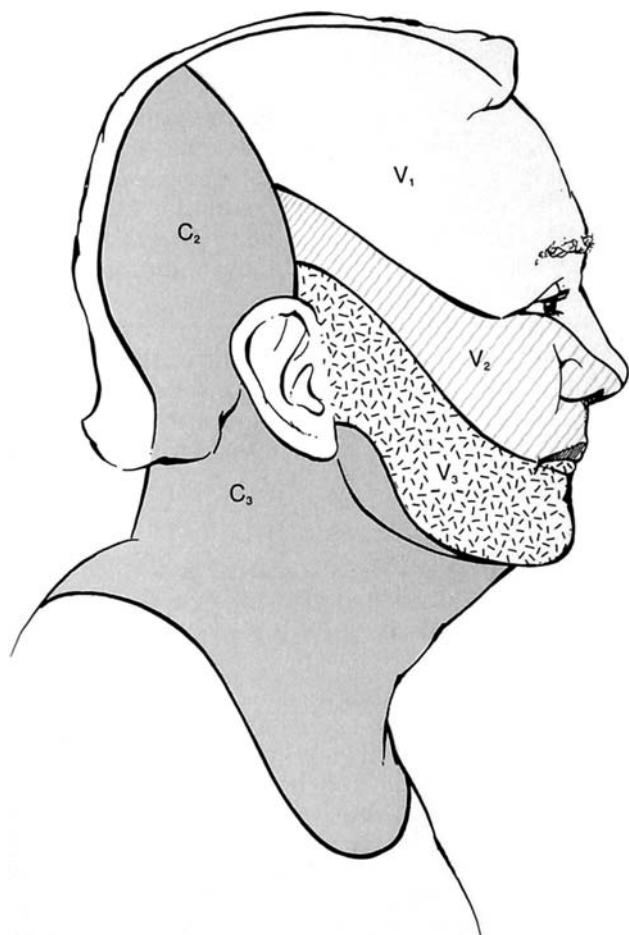


Fig. 2. Sensory fields of the ophthalmic (V_1), maxillary (V_2), and mandibular (V_3) divisions of the trigeminal nerve. The sensory fields of the upper cervical spinal nerves are shown (C_2 , C_3). Note that the skin over the angle of the mandible is innervated by C_2 . See text for description of innervation of the auricle and external auditory canal.

fibers pass through the pterygopalatine ganglion and probably provide some sensory collateral terminals to these neurons. The postganglionic fibers of the pterygopalatine ganglion are functionally associated with the facial nerve, but they accompany the peripheral branches of the maxillary nerve to their targets. For example, one important target of the facial nerve postganglionic parasympathetic innervation is the *lacrimal gland*. These parasympathetic fibers reach the lacrimal gland by following the lacrimal branch of the maxillary nerve. Maxillary nerve peripheral sensory fibers supply maxillary teeth, gingiva, and mucosae of the maxillary sinus, nasal cavity, and palate. Cutaneous fibers follow the infraorbital nerve to innervate skin of the midface, including the lower eyelid, nose, and upper lip (Fig. 2).

2.1.4. THE MANDIBULAR DIVISION OF THE TRIGEMINAL NERVE

The *mandibular nerve* consists of a large sensory component and a smaller motor component. It courses inferiorly from the trigeminal ganglion and exits the middle cranial cavity through the *foramen ovale*. Near the base of the skull, just inferior to the foramen ovale, the parasympathetic postganglionic *otic ganglion* is attached to the medial aspect of the mandibular nerve. The otic ganglion is a component of the parasympathetic outflow of the *glossopharyngeal nerve*, it contains parasympathetic postganglionic neurons destined to innervate the *parotid gland*.

The mandibular nerve conveys sensory innervation from the dura, mandibular teeth and gingiva, mucosae of the floor of the oral cavity, and the anterior two-thirds of the tongue (except taste). Cutaneous branches innervate skin of the chin, lower lip, and cheek (Fig. 2). In conducting an examination of the sensory field of the mandibular nerve, is it important to note that the skin covering the angle of the jaw and the posterior aspect of the scalp is innervated by spinal nerve C_2 .

The *chorda tympani nerve*, a branch of the *facial nerve*, conveys taste sensations from the anterior two-thirds of the tongue and parasympathetic preganglionic innervation to the submandibular ganglion. The submandibular ganglion is located inferior to the tongue and provides postganglionic parasympathetic innervation to the submandibular and sublingual glands. The chorda tympani fibers first follow the *lingual nerve* in the oral cavity inferior to the tongue; the lingual nerve is a branch of the mandibular nerve. Chorda tympani fibers subsequently leave the lingual nerve near the base of the skull and form the *chorda tympani nerve*. It passes through the middle ear cavity just superior to the tympanic membrane and joins the facial nerve in the *facial canal*. The primary sensory cell bodies for taste are in the *geniculate ganglion* associated with the facial nerve; their central processes follow the facial nerve and terminate in the *solitary nucleus*. Although taste fibers accompany the lingual nerve in the floor of the oral cavity, it is important to note that *taste* from the anterior two-thirds of the tongue is a function of the *facial nerve*.

2.1.5. INNERVATION OF THE EXTERNAL EAR

The mandibular nerve provides sensory innervation from the anterior aspect of the *auricle (pinna)*, external surface of the *tympanic membrane*, and most of the *external auditory canal*. Cutaneous branches of

the facial, glossopharyngeal, and vagus nerves also supply small areas of the external auditory canal. The rim of the auricle (helix) and its posterior surface are innervated by spinal nerves C₂ and C₃.

2.1.6. SENSORY INNERVATION OF THE DURA MATTER

The *dura* of the anterior and middle cranial cavities, including the superior aspect of the tentorium, receives sensory innervation from intracranial branches of all three divisions of the trigeminal nerve. Branches of cervical spinal nerves C₂ and C₃, and possibly a small component from the vagus nerve, provide sensory innervation to the dura of the posterior cranial cavity. Pain sensitivities are greatest from the dura along the superior sagittal sinus and from the tentorium.

2.1.7. THE TRIGEMINAL (GASSERIAN OR SEMILUNAR) GANGLION

The neuron cell bodies in the trigeminal ganglion are classified as unipolar (pseudounipolar) primary sensory neurons and vary in size and neurochemical phenotypes (Fig. 1; *see also* Fig. 4). Accordingly, the unmyelinated axons, or C-fibers, originate from the small neurons, whereas larger neurons give rise to myelinated axons. Several subsets of neurons have been described based on their expressions of peptides such as *substance P* (*SP*), *calcitonin gene-related peptide* (*CGRP*), *galanin*, and other peptides (*see Fig. 4A, B*). Approximately 50% of trigeminal ganglion neurons express CGRP and 17% express SP. CGRP and SP are coexpressed by some trigeminal neurons thus providing at least three subsets of neurons based on their content of these peptides. For example, one subset expresses CGRP but not SP, whereas another subset expresses SP but not CGRP. A third subset expresses both peptides; specifically, one-fourth of CGRP neurons also express SP and one-half of the SP neurons coexpress CGRP (*see Quartu et al., 1992*). There are many large sensory neurons that express neither SP nor CGRP (*unstained in Fig. 4*). The physiologic significance of these complex trigeminal neuron chemical phenotypes is not well understood. Many studies have demonstrated that CGRP and SP are present in trigeminal sensory terminals and that these peptides satisfy the criteria necessary for classification as neurotransmitters. These peptides have been implicated in the propagation of nociceptive signaling, inflammatory responses, and other functions.

The unipolar primary sensory trigeminal neurons associated with nonconscious proprioception from

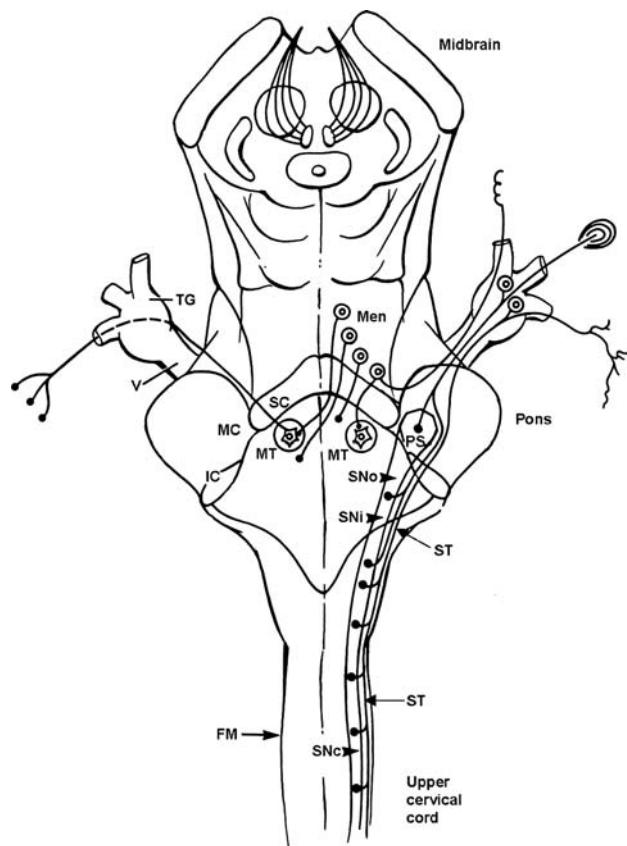


Fig. 3. Posterior view of the brain stem with the cerebellum removed, illustrating the locations of the major components of the trigeminal system. Motor components are represented on the left and sensory components on the right. Double circles represent cell bodies, solid dots represent nerve terminals. The trigeminal ganglion (TG) gives origin to the three divisions of the trigeminal nerve (see text). The central processes of some trigeminal neurons form the spinal trigeminal tract (ST), which lies just lateral to the spinal nucleus (SN) and passes inferiorly through the pons, medulla, into the upper cervical spinal cord. FM, level of the foramen magnum; IC, inferior cerebellar peduncle; MC, middle cerebellar peduncle; Men, mesencephalic nucleus; MT, motor nucleus; PS, principal trigeminal sensory nucleus; SC, superior cerebellar peduncle; SNC, spinal trigeminal nucleus—subnucleus caudalis; SNI, spinal trigeminal nucleus—subnucleus interpolaris; SNo, spinal trigeminal nucleus—subnucleus oralis; ST, spinal trigeminal tract; TG, trigeminal ganglion; V, root of trigeminal nerve.

the muscles of the face are located in the trigeminal mesencephalic nucleus in the pons, not in the trigeminal ganglion (Fig. 3; *see also* Fig. 5D).

2.1.8. MOTOR COMPONENT OF THE TRIGEMINAL NERVE

The *trigeminal nerve* motor component innervates the muscles of mastication, which are the lateral and

medial pterygoid, temporalis, masseter, and mylohyoid muscles, and also innervates the anterior belly of the digastric, tensor veli palatini, and tensor tympani skeletal muscles. It is important to note that the facial and hypoglossal nerves innervate skeletal muscles of the face and tongue, respectively (see Chapter 12).

3. CENTRAL CONNECTIONS OF THE TRIGEMINAL SYSTEM

3.1. Spinal Trigeminal Tract

Most trigeminal sensory A-delta and C-fibers that enter the pons with the trigeminal nerve turn caudally forming the *spinal trigeminal tract* (Fig. 3; *see also* Fig. 5A–C). The spinal trigeminal tract courses just under the lateral surface of the pons and medulla and extends caudally into the upper cervical cord, where it interdigitates with the *dorsolateral fasciculus*. Along its caudal course through the pons and medulla, the spinal trigeminal tract terminals synapse on neurons of the spinal trigeminal nucleus. The spinal trigeminal nucleus lies just medial to the spinal trigeminal tract throughout its course. These fibers carry nociceptive, thermal, and mechanoreceptor stimuli from the ipsilateral face and oral cavity peripheral fields, including gingiva and tooth pulp. Many terminals, especially those containing such peptides as substance P, CGRP, and other peptides associated with thermal and nociceptive transmission, terminate in the *subnucleus caudalis* of the spinal trigeminal nucleus (Fig. 3, Fig. 4A, B, and Fig. 5A, B).

Those sensory fibers that distribute with peripheral branches of the facial, glossopharyngeal, and vagus nerves also join the spinal trigeminal tract and terminate in the spinal trigeminal nucleus. As described earlier, sensory fibers following these cranial nerves provide sensory innervation to parts of the skin of the external auditory canal.

Axons in the spinal trigeminal tract maintain a precise arrangement in their descending course; the ipsilateral face and head are represented in an inverted orientation. Specifically, those fibers originating from the ophthalmic division (upper face) are located most ventral in the tract; maxillary division axons are intermediate; and mandibular division axons, along with those from the facial, glossopharyngeal, and vagus nerves, are positioned posteriorly in the tract.

Some fibers entering the pons with the trigeminal nerve form ascending and descending branches and supply synaptic terminals to both the spinal trigeminal

and the principal sensory trigeminal nuclei. These fibers are probably associated with mechanoreceptors and tactile sensations.

3.2. Trigeminal Nuclei

3.2.1. SPINAL TRIGEMINAL NUCLEUS

The *spinal trigeminal nucleus* is the largest of the trigeminal nuclear components. It extends as a continuous column of small neurons in the lateral tegmentum from mid-pons through the rostral three cervical spinal cord segments (Fig. 3 and Fig. 5A–C). The spinal nucleus receives its most important input from the *spinal trigeminal tract*, which consists of the central processes of some of the sensory neurons in the trigeminal ganglion (Fig. 4C, D). Three spinal trigeminal subnuclei are recognized: *subnucleus oralis* (Fig. 5C), which is anatomically continuous with the principal sensory trigeminal nucleus (Fig. 5C, D); *subnucleus interpolaris* (Fig. 5B) in the mid-medulla and caudal pons; and *subnucleus caudalis* (Fig. 5A) in the caudal medulla. Subnucleus caudalis also extends into the dorsal horn of the upper three segments of the cervical spinal cord where it overlaps *substancia gelatinosa*. The detailed cytomorphology, circuitry, and functions of the trigeminal spinal nucleus, especially the subnucleus caudalis, are similar to those of the *substancia gelatinosa* in the dorsal horn of the cervical spinal cord inferring similar functions in processing nociceptive and thermal sensations.

Subnucleus oralis, the rostral portion of the spinal trigeminal nucleus, receives stimuli originating predominately from the ipsilateral nasal and oral cavities, including dental structures (Fig. 3 and Fig. 5C). The caudal component of the spinal trigeminal nuclear complex, subnucleus caudalis, is functionally related primarily to nociception, general tactile and thermal sensations from the surface of the ipsilateral face (Fig. 3 and Fig. 5A). Specifically, axons innervating the perioral region terminate in the more rostral region of the subnucleus caudalis in the caudal medulla, and sensations from progressively more posterior parts of the face terminate on progressively more caudal portions of the nucleus in the rostral three segments of the cervical spinal cord. This anatomic arrangement explains the *onion skin pattern* of sensory loss occasionally seen as a result of lesions affecting the caudal medulla and upper cervical spinal cord. For example, a tumor exerting pressure on the dorsolateral aspect of the upper cervical cord could interrupt pain and thermal sensations from

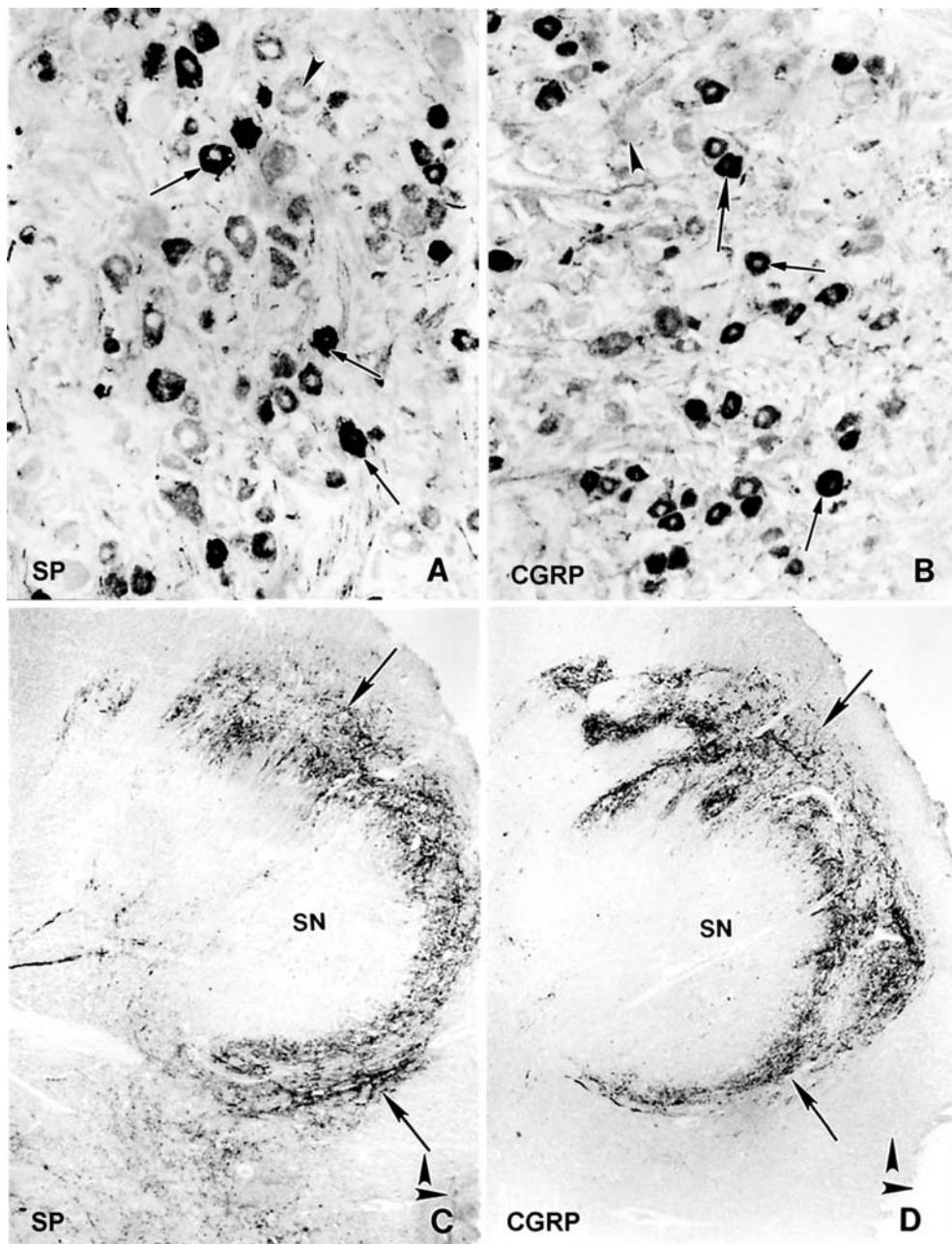


Fig. 4. Immunohistochemical preparations of (A, B) trigeminal ganglion neurons, (C, D) spinal trigeminal nucleus and spinal trigeminal tract. Immunohistochemistry demonstrates (A, C) substance P (SP) and (B, D) calcitonin gene-related peptide (CGRP) in (A, B) human trigeminal ganglia neurons and (C, D) spinal trigeminal nucleus and tract in the human medulla. (A, B) Note that a subset of small and medium-sized trigeminal neurons are intensely immunoreactive for (A) SP or (B) CGRP (arrows). Most large trigeminal neurons are immunonegative (arrowheads). SP- or CGRP-immunoreactive fibers are evident in the background (magnification $\times 140$). (C, D) Note that the central processes and terminals of the trigeminal primary sensory fibers, reaching the superficial layers of the spinal trigeminal nucleus, are intensely immunoreactive for (C) SP or (D) CGRP (arrows). The terminals of these primary sensory fibers will synapse on neurons of the spinal trigeminal nucleus (SN). The axons of the SN neurons will project to the thalamus as the trigeminonthalamic tracts and to other targets (magnification $\times 25$). See Quartu et al., 1992. (Illustrations courtesy of Professor Marina Del Fiacco, University of Cagliari.)

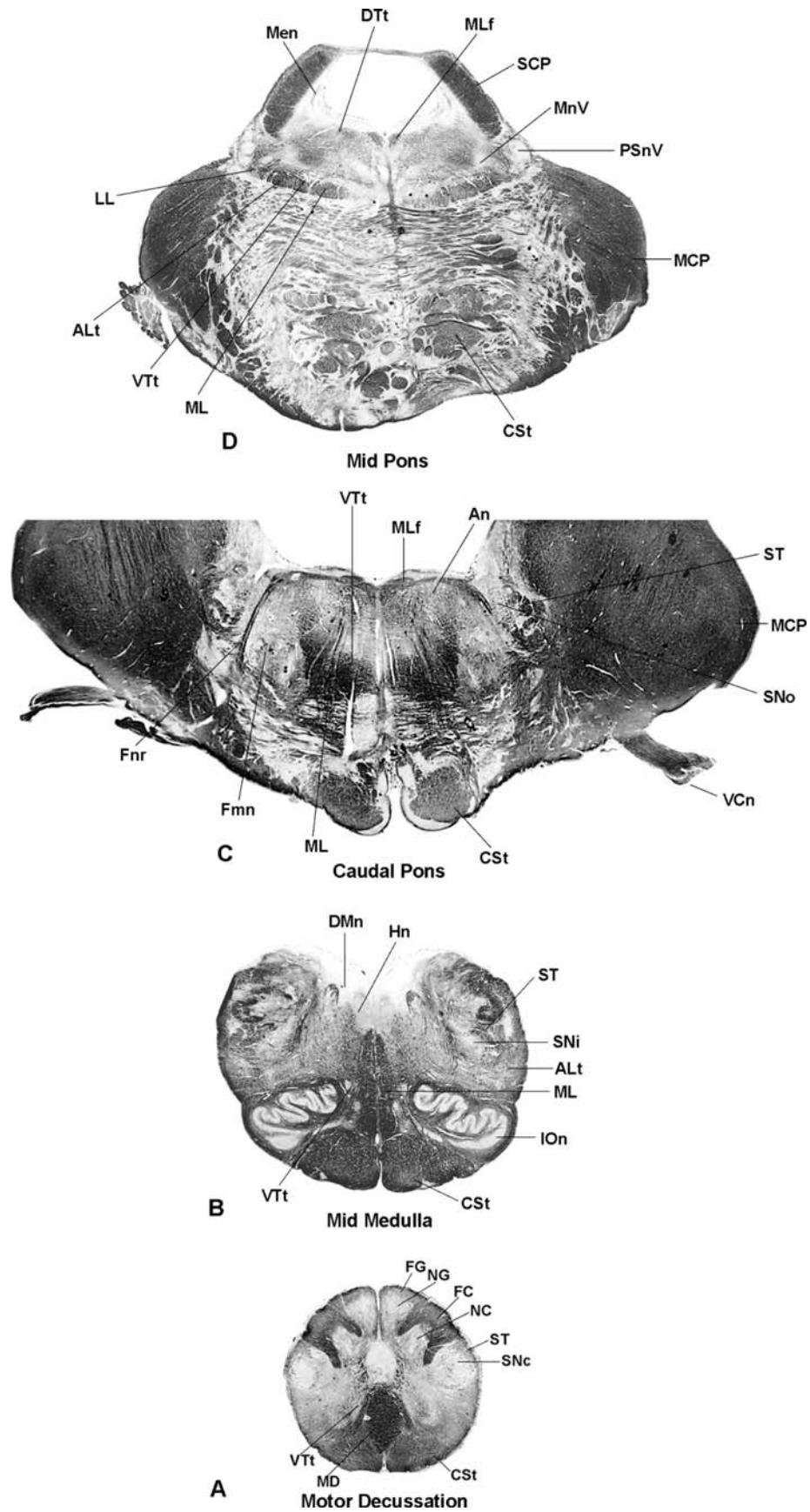


Fig. 5. (Continued)

the posterior portions of the ipsilateral face but spare these sensations in the perioral region.

3.2.2. PRINCIPAL TRIGEMINAL SENSORY NUCLEUS

The *principal sensory nucleus* (also termed chief or superior sensory nucleus of the trigeminal or pontine trigeminal nucleus) receives most of its afferent input from A-beta primary sensory axons, which convey *discriminative tactile, position sense, conscious proprioception, and vibratory sensations* from the sensory fields innervated by all three trigeminal nerve divisions (Fig. 3 and Fig. 5D). Other fibers serving *general (crude) tactile* sensations also terminate in the principal sensory nucleus. Anatomically, the principal sensory nucleus is continuous caudally, and overlaps to some extent, with the subnucleus oralis of the spinal trigeminal nucleus (Fig. 3 and Fig. 5C).

Separation of sensory modalities in the trigeminal nuclei is not sharp. In fact, most neurons of the principal sensory trigeminal nucleus respond to mechanical stimuli in a manner suggesting perception of fine (discriminatory) tactile sensations. In addition, at least some neurons in all sensory trigeminal nuclei respond when mechanical, thermal, or nociceptive stimuli are applied to their receptive fields. Nonetheless, certain nuclear subdivisions respond more prominently to a particular sensory modality. For example, tactile stimuli corresponding with light touch or deflection of hair on the skin activate neurons in the principal sensory trigeminal nucleus and in all subnuclei of the spinal trigeminal nucleus. Most neurons in the principal sensory nucleus and the spinal trigeminal subnucleus oralis are activated by stimuli applied to well-defined sensory fields and conveyed by large-diameter, well-myelinated primary sensory fibers associated with rapidly adapting mechanoreceptors and discriminatory tactile perception. Neurons in the subnucleus caudalis respond best

to thermal or nociceptive stimuli. Prominent inter-nuclear connections between all trigeminal sensory nuclei have been revealed implying that complex integration of sensory modalities occurs in these nuclei.

3.2.3. THE TRIGEMINAL MESENCEPHALIC NUCLEUS

Trigeminal mesencephalic nucleus extends rostrally from mid-pontine levels into the midbrain and lies lateral to the periaqueductal gray (Fig. 3 and Fig. 5D; *see also Chapter 12*). It consists of unipolar primary sensory neuron cell bodies and is the only example of primary sensory neuron cell bodies located in the central nervous system. The peripheral fibers of these neurons are large-diameter, myelinated axons that arise from the mechanoreceptors in the musculature innervated by the trigeminal nerve. In the rostral pons and midbrain, these fibers form the *mesencephalic tract of the trigeminal nerve*. Axons of the *trigeminal mesencephalic nucleus* provide bilateral nonconscious (reflex) proprioceptive input to the trigeminal motor nuclei, the surrounding reticular formation, cerebellum, and to other cranial nerve motor nuclei. The trigeminal mesencephalic nucleus projects proprioceptive information to the cerebellum by way of the *superior cerebellar peduncle*. These connections activate reflex functions associated with chewing, salivation, tongue movements, and swallowing. It should be noted that current evidence suggests that most proprioceptive stimuli originating from extraocular muscles are conveyed by primary sensory neurons whose cell bodies are in the trigeminal ganglion.

3.2.4. TRIGEMINAL MOTOR NUCLEUS

Trigeminal motor nucleus is a well-defined cluster of neurons located in the lateral tegmentum of the mid-pons. It lies ventromedial to principal sensory

Fig. 5. (Continued)

Axial sections of the human brain stem demonstrating the components of the trigeminal system. Nerve fibers stain dark; areas containing mostly neuron cell bodies are relatively unstained. **(A)** Caudal medulla. CSt, corticospinal tracts; FC, fasciculus cuneatus; FG, fasciculus gracilis; MD, motor decussation; NC, nucleus cuneatus; NG, nucleus gracilis; SNC, spinal trigeminal nucleus—subnucleus caudalis; ST, spinal trigeminal tract; VTt, ventral trigeminothalamic tract. **(B)** Mid-Medulla. ALT, anterolateral tracts; CSt, corticospinal tracts; DMn, dorsal motor nucleus of vagus; Hn, hypoglossal nucleus; IOn, inferior olive nucleus; ML, medial lemniscus; SNi, spinal trigeminal nucleus—interpolaris; ST, spinal trigeminal tract; VTt, ventral trigeminothalamic tract. **(C)** Caudal pons. An, abducens nucleus; CSt, corticospinal tracts; Fmn, facial motor nucleus; FnR, facial nerve root; MCP, middle cerebellar peduncle; ML, medial lemniscus; MLf, medial longitudinal fasciculus; SNo, spinal trigeminal nucleus—oralis; ST, spinal trigeminal tract; VTt, ventral trigeminothalamic tract; VCn, vestibulocochlear nerve. **(D)** Mid-pons. Alt, anterolateral tracts; CSt, corticospinal tracts; DTt, dorsal trigeminothalamic tract; LL, lateral lemniscus; MCP, middle cerebellar peduncle; ML, medial lemniscus; MLf, medial longitudinal fasciculus; Men, mesencephalic nucleus; Mvn, trigeminal motor nucleus; PSnV, principal sensory trigeminal nucleus; SCP, superior cerebellar peduncle; VTt, ventral trigeminothalamic tract. *See Chapter 12 for additional labeling of brain stem structures.*

trigeminal nucleus and extends from the abducens nucleus caudally to the level of the inferior colliculus rostrally (Fig. 3 and Fig. 5D). The trigeminal motor nucleus consists mostly of large and some small multipolar neurons. The larger neurons are the *lower motor neurons* for the skeletal muscles innervated by the trigeminal nerve and participate in important reflexes and other responses. The smaller neurons are probably interneurons associated with regulation of trigeminal motor functions.

Corticonuclear projections provide *upper motor neuron innervation* to trigeminal motor neurons; these projections originate in the *face region of the precentral gyrus* and other cortical motor areas. The corticonuclear fibers traverse the *genu of the internal capsule* and the *cerebral peduncle* to reach the trigeminal motor nuclei. There are about equal ipsilateral and contralateral corticonuclear projections to the trigeminal motor nuclei and surrounding reticular formation. Some corticonuclear tract terminals directly innervate trigeminal motor neurons; however, most upper motor innervation is indirect through small internuncial neurons in the surrounding reticular formation. Input to the motor trigeminal nuclei from the trigeminal mesencephalic nuclei are direct and probably forms the sensory limb of the *jaw jerk reflex* (Fig. 3).

Diffuse connections from the hypothalamus and other limbic structures through the reticular formation activate the trigeminal motor nuclei, along with other cranial nerve motor nuclei, to produce the facial expressions that accompany emotional responses. Orofacial reflex activities, such as chewing, swallowing, salivation, and phonation, also involve the trigeminal system and are integrated with other cranial nerves through the reticular formation.

4. ASCENDING TRIGEMINAL PATHWAYS

4.1. Ascending Pathways from the Principal Trigeminal Sensory Nucleus

The principal sensory trigeminal neurons convey tactile and mechanical information via fibers that ascend to the *ventral posterior medial nucleus* of the contralateral thalamus. These fibers form two small tracts, the *ventral (VTTT, trigeminal lemniscus) and dorsal (DTTT) trigeminotthalamic tracts* (Fig. 5 and Fig. 6). DTTT fibers project ipsilaterally to the thalamus whereas VTTT fibers are almost all crossed. DTTT is located in the posterior region of the tegmentum just under the floor of the fourth ventricle. VTTT fibers cross through the tegmentum and ascend in the vicinity of the contralateral medial

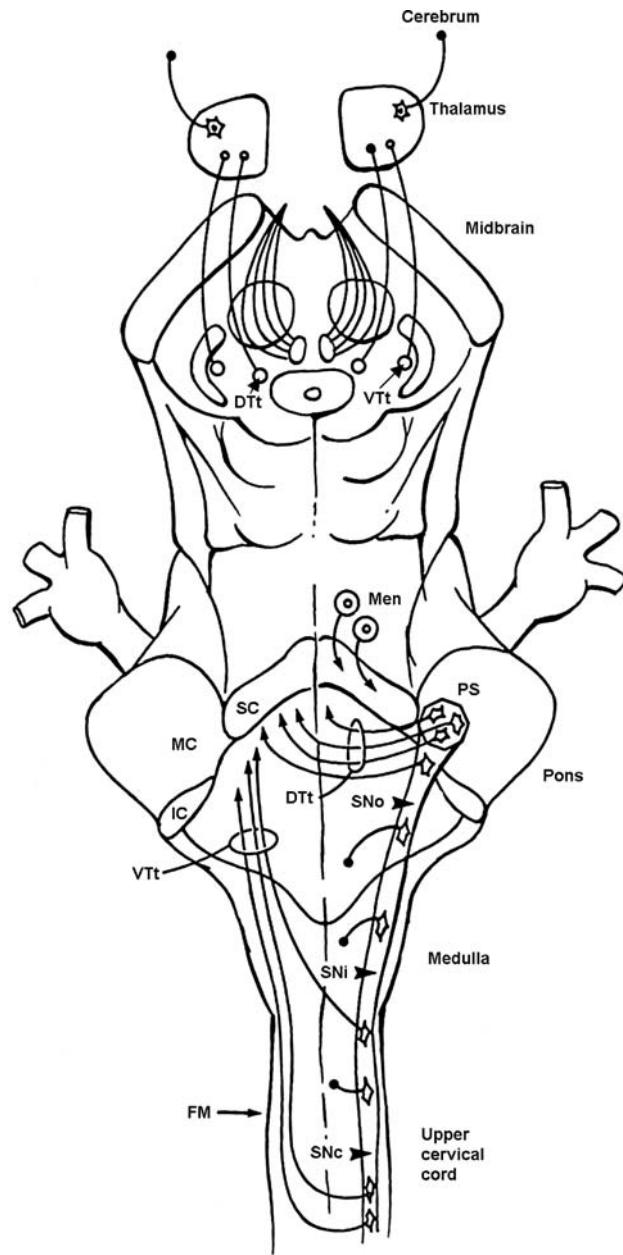


Fig. 6. Posterior view of the brain stem with the cerebellum removed, illustrating the major ascending pathways of the trigeminal system. DTT, dorsal trigeminotthalamic tract; FM, level of the foramen magnum; IC, inferior cerebellar peduncle; MC, middle cerebellar peduncle; Men, mesencephalic nucleus; PS, principal sensory trigeminal nucleus; SC, superior cerebellar peduncle; SNC, spinal trigeminal nucleus—subnucleus caudalis; SNI, spinal trigeminal nucleus—subnucleus interpolaris; SNO, spinal trigeminal nucleus—subnucleus oralis; VTt, ventral trigeminotthalamic tract.

lemniscus to the contralateral thalamus (Fig. 5 and Fig. 6). Thalamocortical fibers project by way of the *posterior limb of the internal capsule* to the *face region of the postcentral gyrus* and other cortical areas.

The cortical projections provide for the conscious appreciation of the character and precise location of tactile and proprioceptive sensations originating in the trigeminal sensory field.

4.2. Ascending Pathways from the Spinal Trigeminal Nucleus

Axons from *spinal trigeminal nucleus*, mostly from *subnucleus caudalis*, cross the midline and collect as the VTTT in the caudal medullary tegmentum near the contralateral medial lemniscus (Fig. 3 and Fig. 5A). VTTT provides collaterals to the reticular formation and ascends to the *ventral posterior medial nucleus* of the thalamus conveying pain, thermal, and some general tactile (i.e., light touch) sensations from the contralateral face. Thalamocortical projections subsequently relay these sensations to the *face region of the postcentral gyrus* for conscious appreciation. It should be noted that all sensory modalities from the face, including taste, are integrated first in the ventral posterior medial nucleus of the thalamus before projection to the postcentral gyrus.

5. FUNCTIONS OF THE TRIGEMINAL SYSTEM

5.1. Modulation of Nociception in the Trigeminal System

As described above, the ascending ventral trigeminothalamic tract conveys nociceptive and other sensory modalities to the thalamus. Thalamocortical projections convey these modalities to the sensory areas of the parietal lobe for conscious appreciation of the location and character of the sensory stimuli. Other ventral trigeminothalamic axons provide collaterals to the *reticular formation* in the medulla and pons and to the *periaqueductal gray* in the midbrain. The reticular formation and periaqueductal gray project to the thalamus, hypothalamus, and limbic structures in the cerebral hemispheres; these circuits are thought to evoke the disagreeable characteristics of pain. The thalamus, hypothalamus, and limbic structures project to the periaqueductal gray and the *raphe nuclei* of the brain stem activating *enkephalinergic and serotonergic neurons*, respectively. This is an essential link in the pain modulating system of the brain. Accordingly, serotonergic neurons of the raphe activate enkephalinergic neurons that inhibit nociceptive transmission through the *subnucleus caudalis*. Other neurochemical circuits are likely involved in the pain modulation effect (see Chapter 21).

5.2. Trigeminal Peripheral Sensory and Motor Distributions Have Important Functional and Diagnostic Implications

Several aspects of the peripheral trigeminal sensory distribution deserve further emphasis. As illustrated in Fig. 2, branches of the ophthalmic division innervate the forehead, anterior scalp, upper eyelid, bridge of the nose, and cornea. Branches of the maxillary division innervate the upper lip, alar region of the nose, most of the cheek, and the lower eyelid. The mandibular division innervates the lower lip, chin, and mandibular region. However, branches of spinal nerves C₂ and C₃ innervate the skin of the angle of the jaw, the posterior scalp, and neck.

The boundaries of the sensory regions innervated by each division of the trigeminal nerve on the face and scalp are sharp and display little overlap with one another or across the midline. In contrast, there is considerable overlap in the sensory dermatome patterns innervated by the spinal nerves on the body surface.

Fibers forming the root and main divisions of the trigeminal nerve follow a course partly in the posterior and middle cranial cavities and thus can be affected by lesions in either of these anatomical compartments.

Discussion of the etiologies of headache is beyond the scope of this chapter, but it should be recalled that most of the dura, falx, and tentorium receive sensory innervation from the trigeminal system. In addition, trigeminal sensory nerves also innervate the major intracranial blood vessels. Intracranial lesions can evoke pain by exerting tension on, or displacing, these structures. However, head pain as perceived by the patient is often not an accurate reflection of the anatomic location or size of the lesion.

A recent analysis of available research data suggests that release of CGRP from trigeminal nociceptor primary sensory neuron terminals may play a role in the generation of migraine headache pain.

5.3. Corneal (Blink) Response Involves Peripheral and Central Components of the Trigeminal and Facial Nerves

The *corneal response* is a normal response to a stimulus applied to the cornea. It is used to test the integrity of the peripheral components of the trigeminal and facial nerves and their internuclear brain stem connections. Here, the examiner touches the cornea with a wisp of cotton, and the normal response is that both eyes blink. The *afferent limb of the corneal response* consists of the primary sensory fibers in the ipsilateral ophthalmic nerve; these fibers terminate on the sensory trigeminal nuclei serving tactile sensations.

Secondary axons arising from the sensory trigeminal nuclei project to both the ipsilateral and contralateral facial motor nuclei in the caudal pons (Fig. 5C). The central connections between the trigeminal sensory nuclei and both facial motor nuclei are through intermediate synapses in the reticular formation. Facial motor fibers follow the facial nerves and innervate their respective orbicularis oculi muscles thereby providing the *efferent limb of the corneal response*. As a result, touching one cornea normally evokes both a direct (in the ipsilateral eye) and a consensual or indirect (in the contralateral eye) responses.

Lesions affecting the ophthalmic or the trigeminal nerves result in no (or diminished) direct and consensual responses when the ipsilateral cornea is touched. However, if the contralateral cornea is touched, both eyes blink because of the bilateral trigeminal sensory input to the intact facial motor nuclei.

The corneal responses are also altered in patients with lesions of the facial motor nucleus or facial nerve. Here, touching the cornea evokes no direct blink response if the ipsilateral facial nucleus or nerve is lesioned, but the contralateral eye does respond because trigeminal sensory input reaches both facial motor nuclei. Alternately, touching the contralateral cornea activates a direct but no consensual response.

Other trigeminal sensory reflexes are activated by sensory trigeminal nuclei through the reticular formation and other pathways. Through these circuits, the trigeminal system participates with other brainstem nuclei in regulating emesis, lacrimation, salivation, swallowing, and other reflexes (Fig. 6).

5.4. Jaw Jerk (Masseter) Reflex Tests Trigeminal Corticonuclear Connections

The *jaw jerk reflex*, an abnormal reflex, is minimally present or absent in normal individuals. It is activated by depressing the mandible or tapping the chin resulting in contractions of the masseter and temporalis muscles. The presence of a jaw jerk reflex is a sign of *hyperreflexia* due to an interruption of the upper motor neuron innervation to the trigeminal motor nuclei via the corticonuclear tracts. The jaw jerk reflex is dependent on proprioceptive input to the trigeminal mesencephalic nuclei and direct, monosynaptic activation of both trigeminal motor nuclei (Fig. 3).

As described earlier, the trigeminal motor nuclei receive about equal bilateral corticonuclear innervation from the face area of the precentral gyri. Accordingly, patients with unilateral lesions affecting the motor cortex, the genu of the internal capsule, or the descending corticonuclear tract in the midbrain

cerebral peduncle have minimal or no observable deficits in jaw movements.

6. TRIGEMINAL SYSTEM LESIONS

6.1. Skull Fractures

Skull fractures involving the petrous portion of the temporal bone can damage the trigeminal nerve as it passes from the posterior cranial cavity to the middle cranial cavity (Fig. 1). In this case, all sensory modalities, except taste, would be lost on the ipsilateral face, tongue, and oral cavity. In addition, opening the mouth would result in deviation of the jaw toward the side of the lesion because of a lower motor neuron paralysis of the ipsilateral muscles of mastication, specifically the lateral pterygoid muscle.

A fracture traversing the foramen ovale on the floor of the middle cranial fossa can damage the mandibular nerve and its motor component but spare functions of the ophthalmic and maxillary nerves. This could result in diminished cutaneous sensations of the ipsilateral lower lip, chin, and jaw region as well as a lower motor neuron paralysis of the ipsilateral muscles of mastication and other muscles innervated by the trigeminal nerve.

In analyzing lesions of the trigeminal nerve or trigeminal motor nucleus, it is important to recall that fibers originating from motor neurons in the facial and hypoglossal nuclei innervate muscles of facial expression and the tongue, respectively, and would not be affected. The sensory modalities from the posterior one-third of the tongue and the oral pharynx are not affected because they are innervated by the glossopharyngeal nerve. Additionally, because the vagus and glossopharyngeal nerves are not involved in this lesion, the gag and swallowing reflexes are intact.

6.2. Space-Occupying Lesions

Space-occupying lesions (e.g., tumors, aneurysms) in the posterior or middle cranial cavities could exert pressure on the trigeminal nerve or its divisions and alter functions in the ipsilateral face (Fig. 1). For example, *cerebellopontine angle tumors* in the posterior cranial cavity (e.g., *acoustic neurinoma* or *schwanoma*) can exert pressure on the lateral aspect of the brain stem and thus on the spinal trigeminal tract and nucleus. Pain and thermal sensations from the ipsilateral face would be altered (either diminished or exacerbated) but tactile sensations, most of which relay in the principal sensory trigeminal nucleus, would be spared (Fig. 3 and Fig. 6). The tumor could also influence the functions of the ipsilateral spinothalamic

tracts, inferior cerebellar peduncle, facial, vestibulocolochlear, glossopharyngeal, and vagus nerves. Alternately, a tumor could exert pressure on the trigeminal nerve; in fact, an early sign of this tumor location is a decreased sensitivity of the ipsilateral cornea resulting in an asymmetric corneal response.

6.3. Trigeminal Neuralgia (*Tic Douloureux*)

Several forms of atypical facial pain syndromes can involve the trigeminal system. One of the more common syndromes is *tic douloureux*, or *trigeminal neuralgia*, which is characterized by sudden onset of excruciating pain lasting for seconds to minutes. The pain is often evoked by tactile stimulation of a particular location, or *trigger zone*, on the face. The trigger zone can be in the perioral region or in the oral cavity. Jaw movements or food in the mouth may evoke bursts of pain. The pain is restricted to the territory of one division of the trigeminal nerve, usually the maxillary, rarely the ophthalmic (Fig. 2). Episodes of pain tend to become more frequent and present a progressively debilitating condition for the patient. Some pharmacologic therapies are helpful, but surgical treatment is usually required for relief. Surgical treatment options include various methods for placing a small lesion in that portion of the trigeminal nerve or ganglion containing the primary sensory fibers or neuron cell bodies innervating the affected region of the face. The lesions can be produced by radiosurgery.

Recently, focused gamma radiation has been used to “lesion” the trigeminal nerve where it enters the pons. This procedure obviates the need for a craniotomy. In some surgical procedures, the neurons that convey pain are more susceptible to the destructive treatment than are the tactile neurons, therefore some tactile sensations are spared on the affected portion of the face. Transection of the spinal trigeminal tract in the caudal medulla is a neurosurgical procedure now rarely used to treat patients with trigeminal neuralgia.

6.4. Pseudobulbar Palsy

Pseudobulbar palsy is due to brain stem vascular lesions that interrupt the corticonuclear tracts (i.e., upper motor neurons) that innervate cranial nerve motor nuclei, including the motor trigeminal nucleus. The resulting weakness (paresis) of skeletal muscles innervated by the cranial nerves interferes with facial, jaw, and tongue movements.

6.5. Posterior Inferior Cerebellar Artery Syndrome

Posterior inferior cerebellar syndrome (PICA; lateral medullary syndrome, Wallenberg's syndrome)

is a cerebrovascular accident involving the intrinsic vasculature supplying the lateral aspect of the medulla (Fig. 5B). It can result in the destruction of several neural structures in the lateral medulla leading to the dysfunctions characteristic of the PICA syndrome. Specific to the trigeminal system, the disruption of blood supply to the spinal trigeminal nucleus and tract results in loss of pain and thermal sensations in the ipsilateral face, but tactile sensations and trigeminal motor function, which depend on the principal sensory trigeminal and trigeminal motor nuclei in the pons, are unaffected (Fig. 3, Fig. 5, and Fig. 6). Other components of the PICA syndrome can include loss of pain and thermal sensations on the contralateral body, ataxia, disequilibrium, dysphonia, weak gag reflexes, dysphagia, and a Horner's syndrome.

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***Marc E. Freeman, David R. Grattan
and Thomas A. Houpert***

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1. INTRODUCTION

1.1. Functions of the Hypothalamus

The hypothalamus is part of the limbic portion of the brain in vertebrates that regulates the *internal milieu* of the cells within narrow limits as it compensates for changing external conditions such as variations in temperature, energy, or defensive requirements. The constancy of the internal environment resulting from these fine adjustments made by the hypothalamus is referred to as *homeostasis*. The hypothalamus maintains homeostasis by exerting control over the two regulatory systems of the organism: the *nervous system* and the *endocrine system*.

In regulating the nervous system, the hypothalamus controls *autonomic processes* such as cardiovascular, thermoregulatory and visceral function. In addition, *behavioral processes* such as ingestive, sexual, maternal, and emotional behaviors are also regulated by the hypothalamus. The role of the hypothalamus in the regulation of the nervous system is summarized in Table 1.

In regulating the function of the endocrine system, the hypothalamus exerts control of the two subdivisions of the *pituitary gland*. The *anterior pituitary* or *adenohypophysis* synthesizes hormones that regulate adrenal, thyroid, and gonadal function as well as

growth and lactation. The synthesis and secretion of anterior pituitary hormones, in turn, are regulated by peptides and amines that are synthesized by and secreted from specific hypothalamic neurons and are transported to the adenohypophysis through a microscopic vascular route, the *hypothalamo-hypophyseal portal system* (Fig. 1) to either stimulate or inhibit the synthesis and secretion of specific hormones of the adenohypophysis. These peptides and amines are known collectively as *releasing* or *release-inhibiting hormones* (Table 2).

The hormones of the *posterior pituitary* or *neurohypophysis* are synthesized by specific hypothalamic neurons and transported to the neurohypophysis axonally by the *hypothalamo-hypophyseal tract* (Fig. 1), released into sinusoids and ultimately into the peripheral circulation to directly regulate blood pressure, water balance, and milk ejection (Table 2).

The fact that certain neurons can subserve two functions, the receipt and transmission of electrical information as typical nerve cells and as endocrine cells that secrete their products into a minute blood supply to regulate the adenohypophysis or into the neurohypophysis and ultimately into the peripheral circulation to regulate visceral processes, has led to the concept of *neurosecretion* and ultimately to the birth of the science of *neuroendocrinology*.

It should be appreciated that hypothalamic control of any one process is not exerted exclusive of other

Table 1
Neural Processes Regulated by the Hypothalamus

Category	System or activity	Process
Autonomic	Cardiovascular	Blood flow (\uparrow or \downarrow) Vasodilation or vasoconstriction
	Thermoregulatory	Blood flow, shivering, panting process
	Visceral	Digestive acid secretion (\uparrow)
Behavioral process	Sexual	Sexual receptivity ("heat")
	Maternal	Nest building
	Emotional	Aggression (\uparrow)
	Ingestive	Eating and drinking (\uparrow or \downarrow)

\uparrow = increase, \downarrow = decrease.

processes. That is, the hypothalamus exerts *integrative function* over physiologic processes. For example, thermoregulatory processes are governed by both the autonomic nervous system and the endocrine system. Exposure to extremes of temperature results in adjustment of blood flow through autonomic processes and metabolic adjustments through regulation of thyroid hormone secretion. Both of these seemingly unrelated controls are under the influence of the hypothalamus. Thus, the hypothalamus can be viewed as the key adaptive part of the brain, ensuring survival processes such as growth, metabolism, reproduction and dealing with the environment.

1.2. Historical Perspective

Though there are indications that the ancients may have appreciated the vital role of higher centers to normal physiology, a role for the hypothalamus did not begin to crystallize until a series of clinical observations made from the late 19th to the early 20th centuries. Most of the early studies focused on hypothalamic control of pituitary function because pituitary pathologies were the most overt. A connection between the hypothalamus and pituitary gland was not appreciated at that time, so many of the early observations were often incorrectly attributed directly to "pituitary tumors." In 1901, Dr. Alfred Fröhlich, a Viennese physician, correctly reported a case of adiposogenital dystrophy in a 14-year-old boy suffering from a pituitary tumor compressing the

optic tract and hypothalamus that was subsequently relieved by surgery. Shortly thereafter, Erdheim described gonadal atrophy and obesity due directly to hypothalamic damage without damage of the pituitary gland. Camus and Roussay (1913) later demonstrated polyuria in dogs bearing surgical lesions of the hypothalamus without damage to the pituitary gland. These were the first direct observations that the hypothalamus controls the pituitary gland. The development of a parapharyngeal procedure to surgically remove the pituitary gland of rats (hypophysectomy) by Philip Smith (1926) led to a flurry of studies of the pituitary gland and brain. In 1932, Moore and Price proposed the important concept of negative feedback mechanisms, describing the reciprocal regulation between the gonad and anterior pituitary gland, although the specific role of the hypothalamus in mediating feedback responses was not fully recognized until much later. It was appreciated at that time that the pituitary gland must remain intact with the brain for coitus to induce ovulation in rabbits. The classic, though albeit, crude experiments of Marshall and Verney (1936) demonstrating ovulation-induction in rabbits by passage of an electrical current through the brain were followed shortly by the experiments of Geoffrey Harris (1937) showing that more localized stimulation of the hypothalamus also led to ovulation induction in rabbits. It was subsequently found by Westman and Jacobsohn (1937) that coitus would not result in ovulation in the rabbit if the pituitary stalk was cut and a foil barrier placed between the hypothalamus and pituitary with the (mistaken) intention of preventing regrowth of severed "nerves." A "fast-forward toward the future" allows us to deduce that coitus stimulated the release of the decapeptide gonadotropin-releasing hormone (GnRH) into portal blood whose role was to stimulate the release of an ovulation-inducing amount of luteinizing hormone into the peripheral circulation.

Possibly the most significant early contribution to the science of neuroendocrinology was the development of the concept of *neurosecretion* by the husband and wife team of Ernst and Berta Scharrer. Beginning in the early 1930s, they proposed that cells of the hypothalamus must have some unique function distinct from other brain cells based on their multinucleated appearance, the abundance of protein-containing colloid-like vacuoles, and the unique proximity between these cells and the surrounding capillary network. The Scharrers proposed that these nerve cells must therefore have a glandular

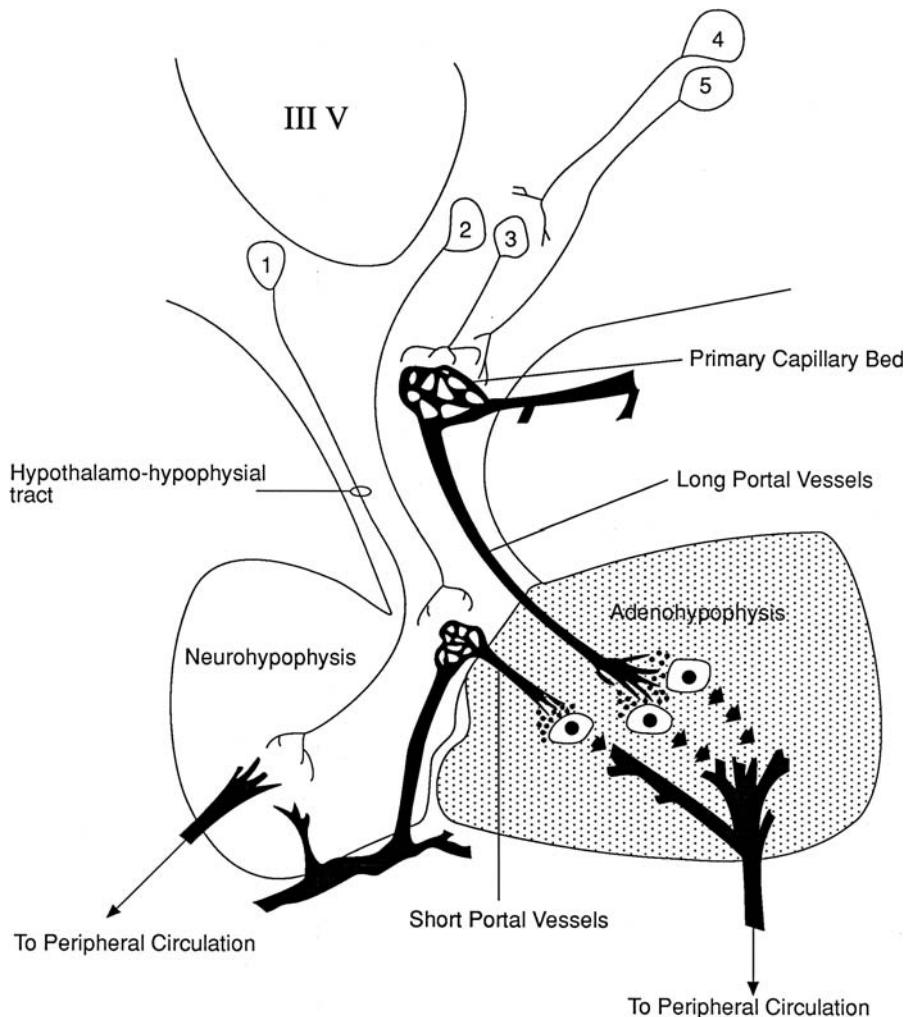


Fig. 1. Diagrammatic representation of the control of the neurohypophysis and adenohypophysis by the hypothalamus. *Neuron 1* is a peptidergic magnocellular neuron from the supraoptic or paraventricular nuclei of the hypothalamus secreting oxytocin or vasopressin into sinusoids in the neurohypophysis. The axons of these two nuclei travel to the neurohypophysis in the hypothalamo-hypophyseal tract. *Neuron 2* could be a hypophysiotropic peptidergic or aminergic neuron terminating adjacent to the short portal vessels, which represent a potential route of communication between the neurohypophysis and adenohypophysis. The hypothalamic release or release-inhibiting peptidergic neurons are of this type. The tuberohypophyseal dopaminergic neurons are also of this type. *Neuron 3* could also be a hypophysiotropic peptidergic or aminergic neuron. In this case, the neuron terminates on the primary capillary bed of the median eminence. It also secretes release or release-inhibiting peptides into portal blood that reach the adenohypophysis via the long portal vessels. The tuberoinfundibular dopaminergic neurons are also of this type. Neurons whose cell bodies lie within the arcuate and periventricular nuclei and terminate on the primary capillary bed in the median eminence compose the infundibular tract. The link between the rest of the brain and the pituitary gland is represented by *neurons 4 and 5*. These are the neurons that secrete catecholamines (and in some cases peptides) that act as neurotransmitters or neuromodulators on the hypophysiotropic neurons. The termination of neuron 4 is axo-dendritic or axo-somatic and that of neuron 5 is axo-axonic.

function. About the same time, Popa and Fielding (1930) described the vascular connection between the hypothalamus and adenohypophysis in rabbits though they mistakenly surmised that the direction of blood flow was from the gland toward the hypothalamus. The first report of flow toward the pituitary was made in toads by Houssay (1935). The

developing concept of neurosecretion coupled with the description of the portal vasculature opened the door for an innovative series of experiments demonstrating that the hypothalamus controlled the adenohypophysis with messages transported over a vascular route. However, experiments involving transection of the stalk connecting the hypothalamus

Table 2
Neuroendocrine Regulators of Hypothalamic Origin

<i>System regulated</i>	<i>Site of regulation</i>	<i>Action</i>	<i>Hypothalamic regulator</i>
Thyroid gland	Adenohypophysis	Stimulation of thyrotropin secretion	Thyrotropin-releasing hormone
Adrenal cortex	Adenohypophysis	Stimulation of adrenocorticotropic secretion	Corticotrophin-releasing hormone
Gonads	Adenohypophysis	Stimulation of luteinizing hormone and follicle stimulating hormone secretion	Gonadotropin-releasing hormone
Muscle, bone liver	Adenohypophysis	Stimulation or inhibition of growth hormone secretion	Growth hormone-releasing hormone, somatostatin
Milk synthesis and secretion from the mammary gland	Adenohypophysis	Inhibition of prolactin secretion	Dopamine
Cardiovascular, renal	Vascular smooth muscle, renal tubule	Vasoconstriction, water reabsorption	Vasopressin, a.k.a. antidiuretic hormone
Mammary gland uterus, and uterus	Smooth muscle of mammary ducts	Increase intramammary pressure inducing milk ejection; increase uterine contraction in labor	Oxytocin

with the pituitary gland led to varying results leading some to doubt the importance of a vascular connection until Green and Harris (1947) suggested that the cut portal vessels could regenerate and Harris (1950) subsequently showed that reproductive function was restored to a degree proportional to portal vessel regeneration in stalk-sectioned rats. Finally, the elegant experiments of Harris and Jacobsohn (1952) convincingly demonstrated the primacy of the hypophyseal-portal vasculature in anterior pituitary function. In these experiments, rats were hypophysectomized and adenohypophyses from their newborns were transplanted to either the temporal lobe of the brain or, by a transtemporal route, immediately beneath the median eminence of the hypothalamus. Only those animals whose transplants beneath the median eminence were revascularized by the portal vasculature showed a resumption of reproductive function. In support of this, Nikitovich-Weiner and Everett (1958) autografted anterior pituitaries to the kidney capsule and demonstrated a loss of thyroid stimulating hormone or thyrotropin (TSH), adrenocorticotropic hormone or corticotropin (ACTH), follicle stimulating hormone (FSH), and luteinizing hormone (LH) secretion but an enhancement of prolactin secretion from the transplants. These transplants were subsequently removed and placed under the temporal lobe of the brain or beneath the median

eminence. Only those rats bearing transplants to the median eminence showed a resumption of normal anterior pituitary function.

The dawning of the science of neuroendocrinology was completed with the focus on the chemical nature of the activities of the adenohypophysis and neurohypophysis. After the studies of Van Dyke and associates (1941) established the existence of separate oxytocic and pressor principals, du Vigneaud identified the structure of oxytocin (1950) and then vasopressin (1954). This was followed by a flurry of work between the mid-1960s through the 1970s by the laboratories of Andrew Schally and Roger Guillemin on the chemical nature of the hypothalamic neuropeptides that control the secretion of TSH, LH/FSH, ACTH, and growth hormone from the anterior pituitary. The “arrival” of the science of neuroendocrinology was recognized by the award of Nobel Prizes to these two investigators in 1977.

2. ANATOMY OF THE HYPOTHALAMUS

2.1. *The Boundaries of the Hypothalamus Are Distinctly Anatomically Defined*

The hypothalamus is situated in the lowermost portion of the *diencephalon* (Fig. 2 and Fig. 3). The human hypothalamus presents well-defined boundaries. The rostral border is limited by a vertical line drawn through the anterior border of the *anterior*

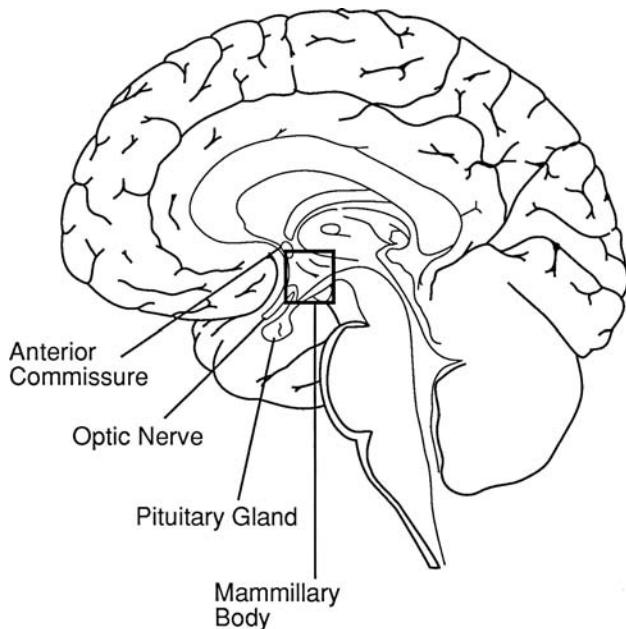


Fig. 2. The position of the hypothalamus and pituitary of the human relative to the rest of the brain. The hypothalamus is bounded by the dark-bordered box.

commissure, lamina terminalis, and optic chiasm. The hypothalamus is bordered caudally by a vertical line drawn through the posterior border of the *mammillary body* as it bounds the *interpeduncular fossa*. The superior border of the hypothalamus is the *hypothalamic sulcus* as it borders the *thalamus*, and the inferior boundary is the bulging *tuber cinereum*, which tapers to form the *infundibulum*, the funnel-shaped functional connection between the hypothalamus and the pituitary gland. Laterally, the borders are ill-defined due to the blending of the hypothalamic gray matter with adjacent structures. However, by convention, the lateral borders are confined by the *internal capsule* and its caudal limits.

2.2. The Divisions of the Hypothalamus Are Described as Functional Groupings

The hypothalamus is divided into clusters of perikarya embedded in gray matter. These are referred to as *nuclei* (singular: *nucleus*). There are several problems inherent in this designation. In most cases, the nuclei are not morphologically distinct structures whose boundaries are distinct in histologic preparation. The dendrites and axons of these neurons may extend for distances beyond the limits of the nucleus. Moreover, chemically and functionally the nuclei may be heterogeneous to varying degrees. Thus one can only infer vague functional and anatomic boundaries for hypothalamic nuclei.

One can describe the groupings of the nuclei in a *rostro-caudal* direction as (1) *anterior or supraoptic* (Fig. 4), located between the lamina terminalis and the posterior edge of the optic chiasm; (2) *medial or tuberal* (Fig. 5), located between the optic chiasm and the mammillary bodies; and (3) *posterior or mammillary* (Fig. 6), including the mammillary bodies and the structures just dorsal to them. One can also describe the hypothalamus longitudinally in mediolateral zones (Fig. 4, Fig. 5, and Fig. 6) known as (1) *periventricular*, bordering the third ventricle; (2) *medial*, comprising the major hypothalamic nuclei that are sites of limbic system projections; and (3) *lateral*, which is separated from the medial zone by the *fornix*, a large C-shaped tract that interconnects limbic system structures.

The most anterior hypothalamic areas are ill-defined. Rather than being grouped as diencephalic structures, the *medial preoptic* and *septal* areas are actually part of the telencephalon (Fig. 4A). However, modern embryology has shown that the preoptic area has the same embryonic origins as many diencephalic structures. Thus, the preoptic area is often considered part of the hypothalamus, but the consensus is not overwhelming. The *medial preoptic area* (Fig. 4A) has been shown to possess sexually dimorphic features. As described later, uniquely stained groups of neurons form in this area in organisms exposed to testosterone prenatally or neonatally. The *lateral preoptic area* (Fig. 4A) is not morphologically distinct from the medial preoptic area but subserves uniquely distinct physiologic roles. More caudally the *anterior* and *lateral hypothalamic areas* appear (Fig. 4B). The cells of these areas are small with few dendritic branches. The lateral hypothalamic area receives fibers from the medial forebrain bundle. Chemical lesion of this area leads to aphagia. As discussed later, this area plays a stimulatory role in feeding behavior. The *paraventricular nucleus* (Fig. 5A) is wedge-shaped and, as its name implies, lies adjacent to the third ventricle. The deeply staining neurons are of two types: *magnocellular* or neurons with large perikarya (magno = large) and *parvicellular* or neurons with small perikarya (parvi = small). The axons of the magnocellular neurons terminate in the neurohypophysis whereas the axons of the parvocellular neurons terminate on the primary capillary bed of the hypophyseal portal vasculature in the median eminence. The *supraoptic nucleus* is found directly above the beginning of the optic tracts and consists of a large anterolateral subnucleus and a smaller posteromedial subnucleus connected by a

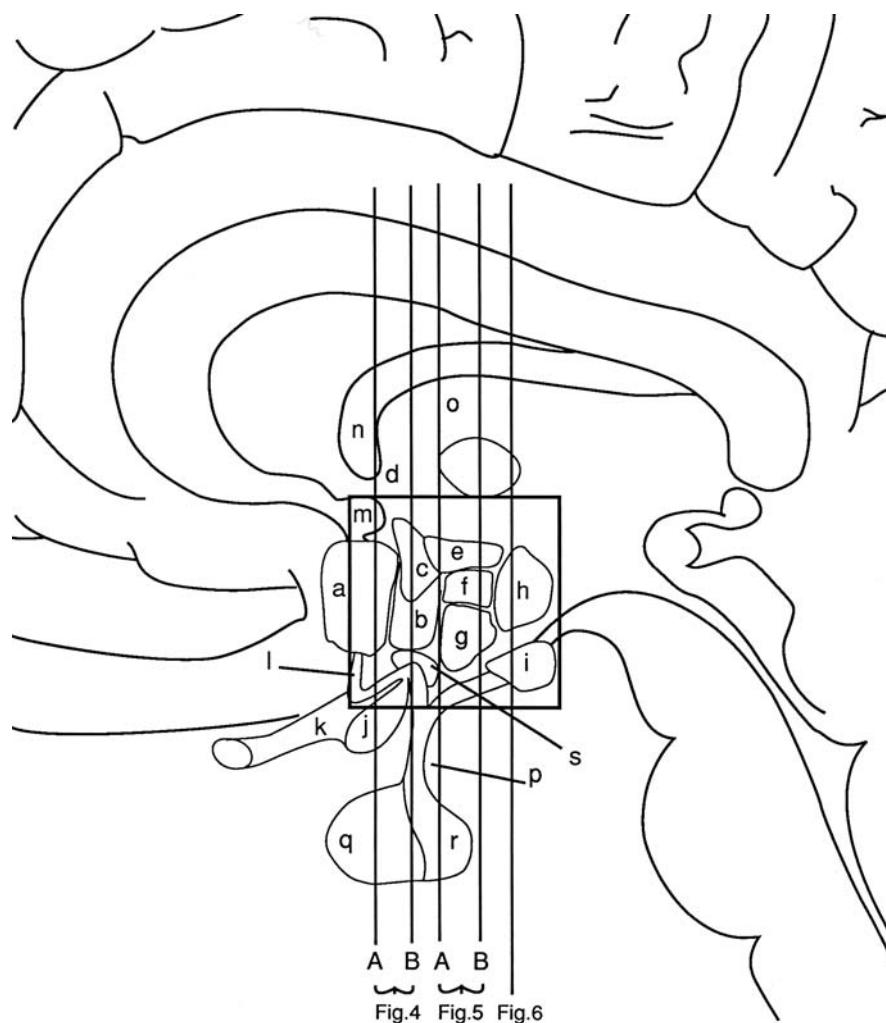


Fig. 3. The position of hypothalamic nuclei and adjacent structures in sagittal section. The vertical lines represent the planes of frontal Fig. 4A, B, Fig. 5A, B, and Fig. 6. The box outlines the corresponding area of Fig. 2. a, preoptic nucleus; b, anterior hypothalamic area; c, paraventricular nucleus; d, hypothalamic sulcus; e, dorsal hypothalamic area; f, dorsomedial nucleus; g, ventromedial nucleus; h, posterior hypothalamic area; i, mammillary body; j, optic chiasm; k, optic nerve; l, lamina terminalis; m, anterior commissure; n, fornix; o, thalamus; p, infundibulum; q, adenohypophysis; r, neurohypophysis; s, supraoptic nucleus.

thin strand of cells (Fig. 5A). As in the paraventricular nucleus, the neurons of the supraoptic nucleus stain darkly and consist of magnocellular perikarya whose axons terminate in the neurohypophysis. The axons of the supraoptic and paraventricular nuclei travel in a bundle, the *hypothalamo-hypophyseal tract*, to the neurohypophysis (Fig. 1). The magnocellular and parvcellular cells of these regions produce vasopressin and oxytocin. The *suprachiasmatic nuclei* are distinctly staining paired structures (in rodents) overlying the *optic chiasm*. In man, the suprachiasmatic nuclei are not strikingly morphologically distinct. In all mammals, the cells of this area receive retinohypothalamic input and are believed to be the

“circadian clock” that controls the temperature cycle, sleep/wake cycle, and the circadian changes in the timing of certain hormone systems such as those pituitary hormones that control the adrenal cortex (ACTH) and the reproductive system (LH and prolactin). The *periventricular nuclei* (Fig. 5) have small perikarya that contain some of the release and release-inhibiting factors controlling the pituitary gland. Also associated with the anterior hypothalamic area are found the morphologically indistinct telencephalic structures known as *circumventricular organs*. One of these is the *organum vasculosum of the lamina terminalis (OVLT)* and the other is the *subfornical organ*. These are areas where the blood-brain

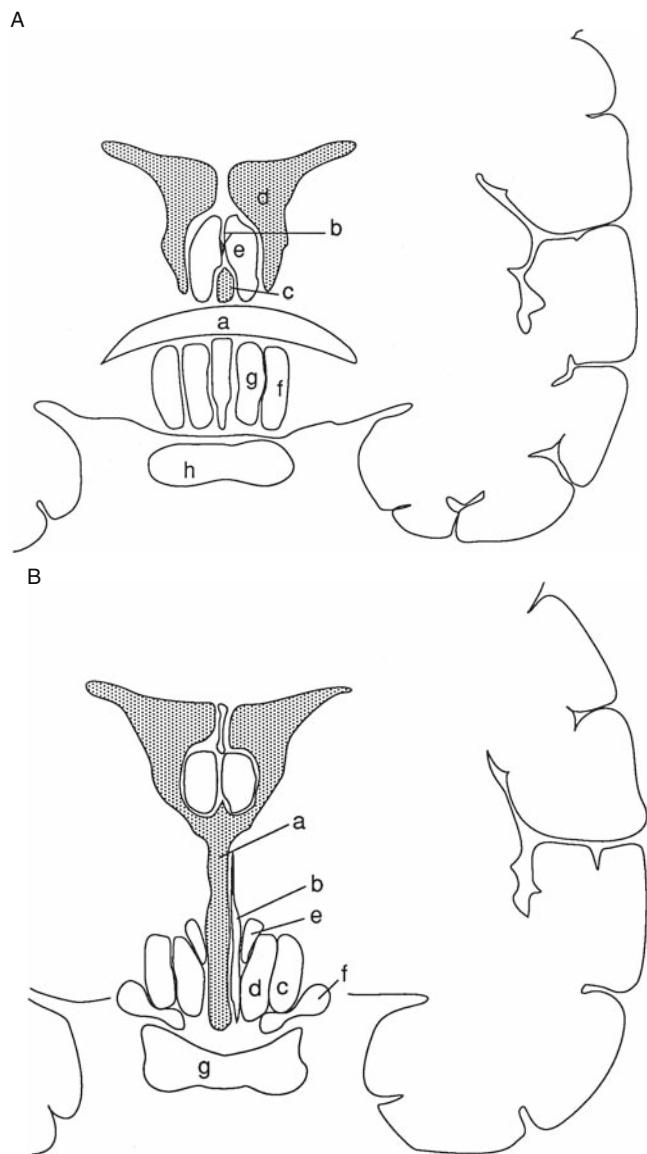


Fig. 4. The hypothalamic and adjacent structures of the anterior or supraoptic groupings. (A) a, anterior commissure; b, septal area; c, third ventricle; d, lateral ventricle; e, column of fornix; f, lateral preoptic area; g, medial preoptic area; h, optic chiasm. (B) a, third ventricle; b, periventricular nucleus; c, lateral hypothalamic area; d, anterior hypothalamic area; e, paraventricular nucleus; f, supraoptic nucleus; g, optic chiasm.

barrier is absent and thus can sense plasma osmolality. These areas play a role in regulation of blood pressure and thirst as they contain angiotensin II receptors and may even be able to synthesize their own angiotensin II. The OVLT has also been implicated in the control of LH secretion by GnRH. The other “leaky” circumventricular organs are the *subcommissural organ* and the *area postrema*.

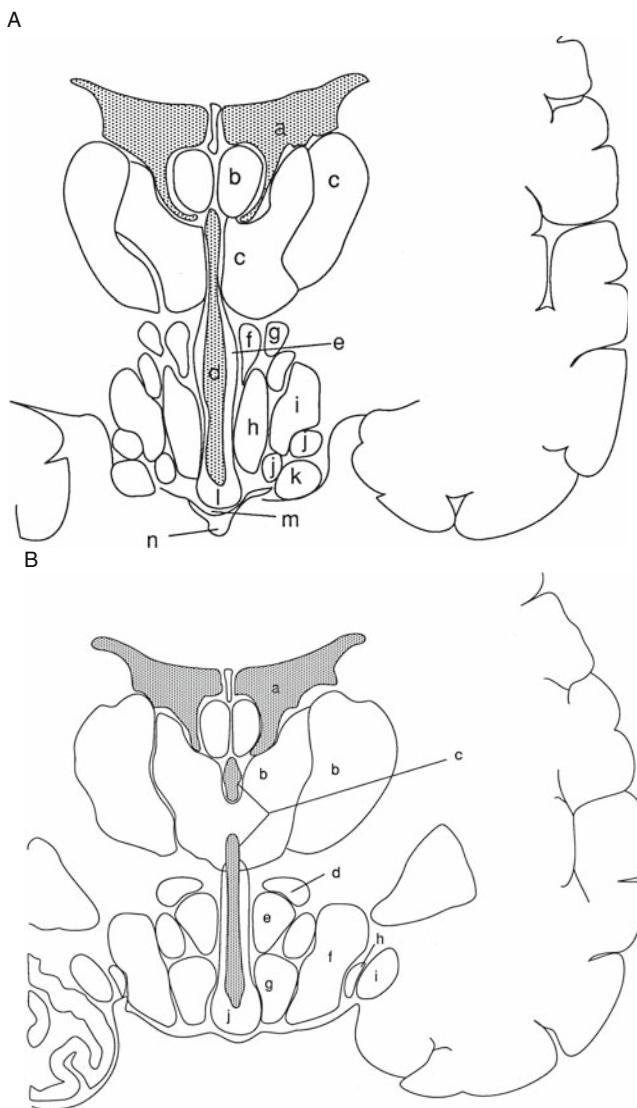


Fig. 5. The hypothalamic and adjacent structures of the medial or tuberal groupings. (A) a, lateral ventricle; b, body of fornix; c, thalamus; d, third ventricle; e, periventricular nucleus; f, paraventricular nucleus; g, dorsal hypothalamic area; h, anterior hypothalamic area; i, lateral hypothalamic area; j, supraoptic nucleus; k, optic tract; l, arcuate nucleus; m, median eminence; n, infundibulum. (B) a, lateral ventricle; b, thalamus; c, third ventricle; d, dorsal hypothalamic area; e, dorsomedial nucleus; f, lateral nucleus; g, ventromedial nucleus; h, supraoptic nucleus; i, optic tract; j, arcuate nucleus.

In the medial area, the optic tracts separate, the lateral hypothalamus continues caudally, and the caudal termination of the supraoptic nucleus is found. Also in this area, the anterior hypothalamic area ends (Fig. 5A) and is replaced by two distinct nuclei, the *dorsomedial* and *ventromedial nuclei* (Fig. 5B). The two can be separated by the small cells of the dorsomedial nucleus and the dense

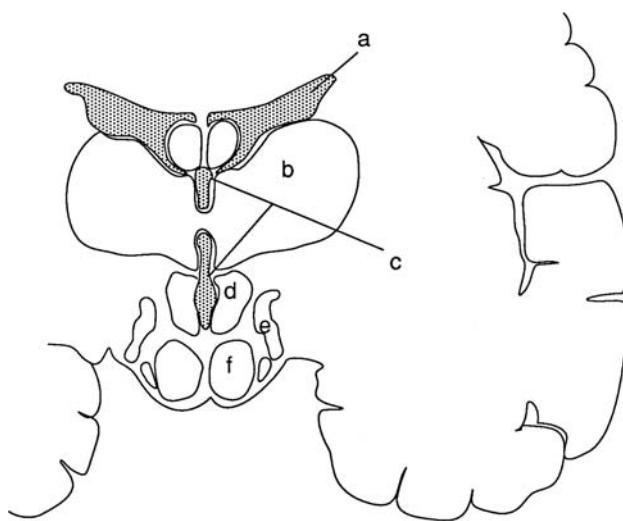


Fig. 6. The hypothalamic and adjacent structures of the posterior or mammillary groupings. a, lateral ventricle; b, thalamus; c, third ventricle; d, posterior hypothalamic area; e, lateral hypothalamic area; f, mammillary body.

grouping of the neurons in the ventromedial nucleus. Both of these nuclei play a role in food intake. Lesions of both nuclei cause hyperphagia. Thus, as detailed later, this area regulates food intake in an inhibitory manner. The ventromedial nucleus in particular contains glucose-sensitive cells that are believed to be the site through which caloric intake is monitored. The cells of the ventromedial nucleus are also rich in receptors for the gonadal steroids estrogen and testosterone and thus are believed to play a major role in reproductive behavior and regulation of hormone secretion from the adenohypophysis. Finally, the *arcuate nucleus* begins in this medial hypothalamic area (Fig. 5). The parvicellular neurons in this area have short axons; some of which terminate on the *primary capillary bed* of the hypothalamo-hypophyseal portal system. The primary capillary bed is found in the underlying *median eminence* (Fig. 5). The neurons of the arcuate nucleus have several functions. In some mammals such as guinea pig, human, most monkeys, bats, ferrets, cows, horse, cat, dog, and rabbit, some GnRH neurons are in the basal portion of the medial hypothalamus. However, in others such as the rat and sheep, this area is devoid of GnRH neurons or small numbers of such neurons are found in a so-called *cell-poor zone*. In those species in which the arcuate nucleus contains GnRH neurons, the fibers continue to the median eminence with some continuing through the infundibular stalk and into the neurohypophysis (human). A second function is in the control of

prolactin and growth hormone secretion. This area is populated by cells that contain dopamine, the prolactin-inhibiting hormone, and growth hormone-releasing hormone, the peptidergic stimulator of growth hormone secretion. As described in Section 4, the arcuate nucleus is also a key center in the regulatory control of body weight, with several neuronal populations involved in the stimulation (NPY/AGRP) or inhibition (α MSH) of appetite and food intake. Finally, the arcuate nucleus is abundant in cells that contain β -endorphin, the endogenous opioid that is a cleavage product of the larger peptide, proopiomelanocortin. These neurons project to various hypothalamic and forebrain sites and are believed to play a role in emotional behavior as well as endocrine function.

The posterior hypothalamic area contains the continuation of the lateral hypothalamic area as well as the *posterior hypothalamic nuclei* and *mammillary bodies* (Fig. 6). The posterior hypothalamic nucleus contains both small and large cell bodies that give rise to efferent fibers descending through the central gray matter as well as the reticular formation of the brain stem. It is believed that these neurons play a role in temperature regulation as they respond to cooling with the induction of shivering as well as the burning of brown adipose tissue. The mammillary nucleus is actually a complex consisting of medial and lateral nuclei. The mammillary bodies are critical circuits linking the hypothalamus with the limbic forebrain and midbrain structures lying rostral and caudal, thus implying a role in hypothalamic activity.

2.3. The Afferent and Efferent Connections Are the Information Pathways of the Hypothalamus

The afferent and efferent connections of the hypothalamus reveal that this part of the brain is a complex integration center for somatic, autonomic, and endocrine functions.

2.3.1. INTRINSIC TRACTS

There are two main intrinsic tracts in the hypothalamus (Fig. 1). The *infundibular tract* arises from neurons in the arcuate nucleus and periventricular nucleus with terminals on capillaries within the median eminence. These axonally transport substances such as dopamine to the portal vessels. The *hypothalamo-hypophyseal tract* arises in the supraoptic and paraventricular nuclei and terminates in the neurohypophysis. As noted earlier, these axons transport vasopressin and oxytocin, respectively. Both of

these transfer information unidirectionally, from the hypothalamus to the pituitary gland.

2.3.2. EXTRINSIC TRACTS

The lateral hypothalamus is reciprocally connected with the *thalamus*, the *paramedian mesencephalic area (limbic midbrain area)*, and the *limbic system*. The medial hypothalamus also receives connections from the limbic system (Fig. 7). It is quite clear that higher cortical centers communicate with the hypothalamus through the limbic system. In addition to the hypothalamus, the limbic system includes the *hippocampus*, the *amygdala*, the *septal area*, the *nucleus accumbens* (part of the *striatum*), and the *orbitofrontal cortex*. Anatomically, the hypothalamus is intimately related to the amygdala, which sits in the temporal lobe just rostral to the hippocampus. Efferents from the amygdala enter the hypothalamus via the *ventral amygdalofugal pathway*. The rostral amygdalofugal fibers form the *diagonal band of Broca*. More caudally, these fibers fan out and enter the hypothalamus, many of which terminate near the ventromedial nucleus. A second afferent to the hypothalamus arises from the *corticomedial amygdala*. This pathway, the *stria terminalis*, terminates near the ventromedial nucleus of the hypothalamus. The other major limbic afferent to the hypothalamus arises from the hippocampus. The body of the hippocampus gives rise to the columns of the *fornix*, which courses toward the *anterior commissure* and then splits into two portions. The *postcommissural fornix* terminates in the mammillary bodies at the caudal end of the hypothalamus. Arising from the mammillary bodies is the

mammillothalamic tract, which goes to the anterior nuclei of the thalamus and from there projects to the cingulate gyrus, the parahippocampal gyrus, and then back to the hippocampus. A second efferent projection from the mammillary bodies, the *mammillotegmental tract*, turns caudally to the ventral tegmentum. A reciprocal pathway from the ventral tegmentum to the mammillary bodies is the *mammillary-pudendal tract*. The *dorsal-longitudinal fasciculus of Schutz* are efferents from the periventricular nuclei of the hypothalamus that terminate in the mesencephalic central gray. Stimulation of this fiber bundle produces fear and aversive reactions. There is a subset of ganglion cells in the retina that project to the suprachiasmatic nucleus of the hypothalamus by way of the *retinohypothalamic tract*. This tract transmits lighting periodicity information to be transduced by the suprachiasmatic nucleus. The *hypothalamospinal tract* originates in the supraoptic and paraventricular nuclei (parvicellular division), which projects down the spinal cord to the thoracic level and terminates in the intermediolateral column and from there to the preganglionic sympathetic nerves. Based on the anatomy, it is obvious that this pathway must be important as the pathway over which the hypothalamus influences autonomic function. The other major hypothalamic fiber tract is the *median forebrain bundle*. This is a collection of tracts with ascending and descending fibers that run in the lateral hypothalamus between the midbrain reticular formation and the basal forebrain. The descending fibers originate from structures in the basal forebrain including the olfactory cortex, the preoptic area, the

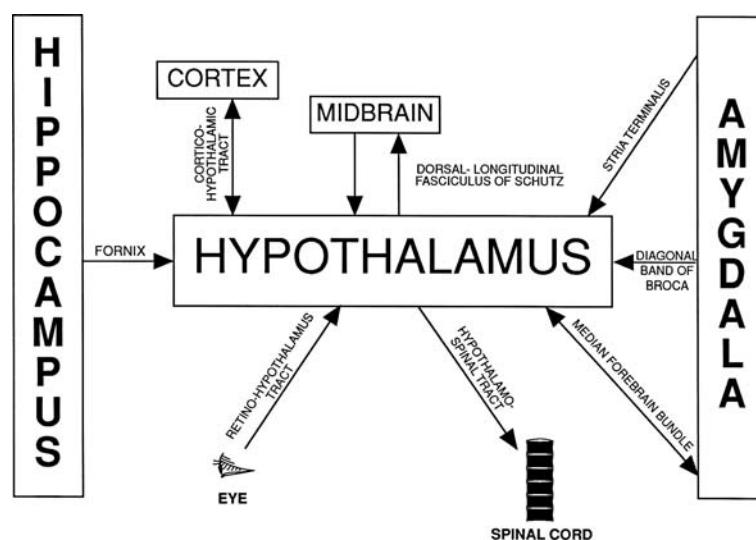


Fig. 7. A diagrammatic representation of some afferents and efferents of the hypothalamus.

septal area, the accumbens, and the amygdala. The ascending portion comes from spinal cord and reticular formation, visceral and taste nuclei in the brain stem, as well as monoaminergic centers in the brain stem.

2.4. Blood Flow as a Means of Communicating Hypothalamic Information

The key neurohumoral link between the hypothalamus and the pituitary gland is the *hypothalamo-hypophyseal portal vasculature* (Fig. 8). It arises from a *primary capillary plexus* that extends from the median eminence to the adenohypophysis. This plexus is supplied with blood from three sources: rostrally by the *superior hypophyseal artery*, caudally by the *inferior hypophyseal artery*, and mediorostrally

by the *anterior hypophyseal artery* (*or trabecular artery*). All three of these arise from the *internal carotid artery*. In some species (rat, rabbit, and cat) they unite to form a single artery that supplies the infundibular stem. These arteries encircle the median eminence. The inferior hypophyseal artery also supplies the neurohypophysis. The primary capillary plexus in the median eminence is drained by the fenestrated *long portal vessels*, which course to the adenohypophysis where they branch to a *secondary capillary plexus*. The primary capillary plexus is the site at which axon terminals converge to release their quanta of hypophysiotropic peptides into portal blood. After transport through the long portal vessels, they are released from the secondary capillary plexus to the surrounding cells of the adenohypophyseal cells. A set

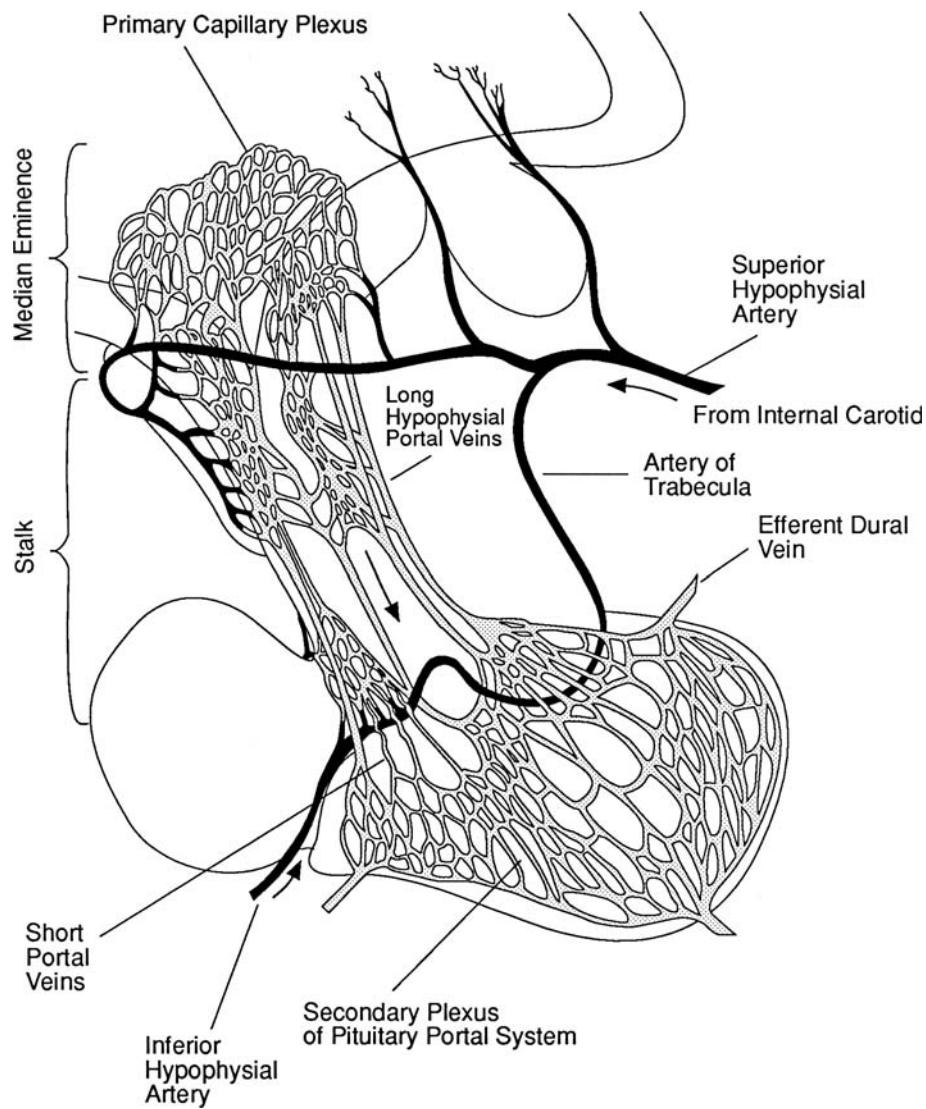


Fig. 8. A schematic representation of the hypothalamo-hypophyseal portal vasculature in man. See text for details.

of *short portal vessels* arise from the anterior hypophyseal artery. These connect the infundibular stem, the neurohypophysis, and the intermediate lobe of the pituitary gland to the adenohypophysis. The short portal vessels are the route over which neurohypophyseal and intermediate lobe peptides travel to the anterior pituitary.

2.5. The Chemiarchitecture Describes the Functions of the Hypothalamus

As described earlier, the neuroendocrine regulatory role was the first characterized function of the hypothalamus. This neuroendocrine function is classically represented by the magnocellular neurosecretory neurons of the posterior pituitary gland, which produce and secrete the hormones oxytocin and vasopressin. Together with the neurons that synthesize and release the hypophysiotropic hormones to regulate secretion from the anterior pituitary gland, these peptide-secreting neurons define the neuroendocrine functions of the hypothalamus. In addition to these hypothalamic hormones, specific hypothalamic neurons also produce a range of other bioactive peptides, which are often coexpressed with classic neurotransmitters including gamma-aminobutyric acid (GABA) and glutamate. These neuropeptides have a modulatory role on hypothalamic function, regulating the activity of the neurosecretory neurons, and also controlling a range of autonomic and behavioral functions (Table 3).

2.5.1. IDENTIFICATION OF HYPOTHALAMIC PEPTIDES

There are time-honored steps that must be taken to identify peptides as physiologically significant in the hypothalamus (Table 4). First, a quantitative bioassay must be established. A specific, dose-dependent

Table 4
Strategies in the Analysis of a Hypothalamic Neuropeptide

1. Development of quantitative bioassay
2. Proof of peptidic nature
3. Development of extraction scheme
4. Chemical and physical characterization
5. Synthesize peptide and determine its bioactivity
6. Produce antibodies to the peptide
7. Use antibodies for immunocytochemical localization and radioimmunoassay
8. Isolate the cDNA encoding the precursor of the peptide

relationship must be established between amount of peptide and the biological response. Second, evidence must be provided that the biologically active material is peptidic in nature. This can be established by demonstrating that proteolytic enzymes diminish or destroy the biological activity. Third, a scheme for extraction and separation of maximal yields of the purified peptide must be devised. Fourth, chemical and physical characterization of the peptide must be performed. This would consist of molecular weight characterization as well as amino acid composition and sequencing. Fifth, once the sequence is known, the peptide must be synthesized and the synthetic product tested for biological activity in the bioassay. Sixth, antibodies to the peptide must be produced, and the purified antibodies must be characterized using synthetic analogues of the peptide. Seven, immunologic approaches with the antibodies must be developed. This would consist of immunohistochemistry for visualization of peptides in neural tissue

Table 3
Classes of Hypothalamic Peptides

Class	Function	Example
1. Hypophysiotropic peptides	Regulate adenohypophysis	TRH, GnRH, GHRH, CRH, somatostatin
2. Neurohypophyseal peptides	Regulate water retention blood pressure, milk ejection,	Vasopressin, oxytocin
3. POMC-derived peptides	Neuromodulatory, neuroendocrine	Endorphins, ACTH
4. Opioid peptides	Neuromodulatory, neuroendocrine	Dynorphin, endorphins, enkephalin
5. Brain-gut peptides	Neuromodulatory, neuroendocrine	VIP, CCK, substance P
6. RF-amides	Neuromodulatory, neuroendocrine	Kisspeptin, prolactin-releasing peptide, gonadotropin-inhibitory hormone
7. "Other" peptides	Neuromodulatory, neuroendocrine	Angiotensin II, NPY, galanin, endothelins, orexin

as well as radioimmunoassay for quantitation of the concentration of the peptide in neural tissue and portal blood. Finally, the cDNA that encodes the precursor of the peptide must be isolated and methods such as *in situ* hybridization histochemistry and Northern blotting developed for detecting the mRNA of the precursor. Most of these approaches have been taken to identify the peptides of importance to hypothalamic function.

2.5.2. NEUROHYPOPHYSEAL HORMONES

Arginine-vasopressin (*AVP*), which is also known as *antidiuretic hormone (ADH)*, and another neurohormone, *oxytocin (OT)* (Fig. 9), are produced in magnocellular neurons whose cell bodies are located in the supraoptic and paraventricular nuclei of the

hypothalamus and whose axons project to the posterior pituitary gland (Fig. 10; Table 5). The large cell bodies (i.e., magnocellular) reflect the extremely high synthetic capacity of these neurons, as required to synthesize sufficient hormone to release into the systemic circulation and act on targets distant from the site of production (i.e., kidneys for AVP and smooth muscle of the reproductive tract for OT). The hormones are synthesized in the nerve cell bodies within the hypothalamus as *prohormones*, or precursor proteins (Fig. 11). These are large molecules that consist of packaging peptides and specific axonal transport peptides, *neurophysins (NP)*, as well as the bioactive nonapeptide (AVP or OT) that is ultimately found in the peripheral circulation. There are actually two forms of NP. NP-I or estrogen-linked neurophysin

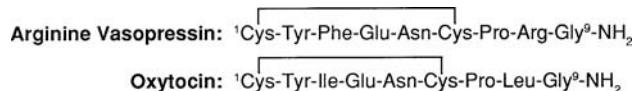


Fig. 9. Amino acid sequences of the *neurohypophyseal peptide* hormones. Note that they share common features: both are nonapeptides, have an intramolecular disulfide bond, and are amidated at the carboxyl terminal. The differences that account for their differing bioactivities are found at positions 3 and 8. In addition, a closely related *pressor peptide*, lysine vasopressin, differs from AVP by substituting lys for arg in position 8.

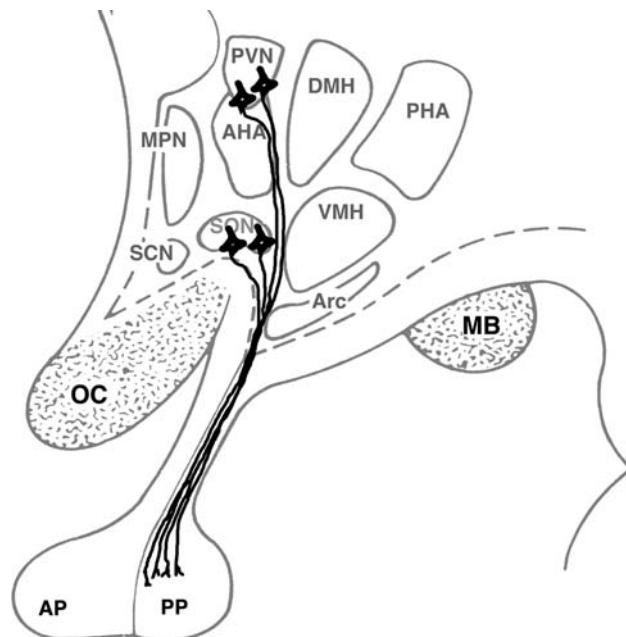


Fig. 10. Diagrammatic representation of a sagittal section through the hypothalamus, showing the distribution of magnocellular neurosecretory neurons that secrete OT and AVP in the posterior pituitary (MPN, medial preoptic nucleus; PVN, paraventricular nucleus; AHA, anterior hypothalamic area; DMH, dorsomedial hypothalamic nucleus; PHA, posterior hypothalamic area; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; VMH, ventromedial hypothalamic nucleus; Arc or Arn, arcuate nucleus; OC, optic chiasm; MB, mammillary bodies; AP, anterior pituitary; PP, posterior pituitary). The cell bodies are located in the supraoptic nucleus and the magnocellular part of the paraventricular nucleus. Axons form the hypothalamo-hypophyseal tract to the posterior pituitary (neurohypophysis).

Table 5
Localization of Neurohypophyseal Peptides

Peptide	Cell bodies	Fibers	Terminals	Presence in portal blood
OT	Paraventricular nucleus, supraoptic nucleus	Hypothalamo-hypophyseal tract	Neurohypophysis, median eminence	+
AVP	Supraoptic nucleus, paraventricular nucleus, suprachiasmatic nucleus, bed nucleus of stria terminalis, nucleus of diagonal band of Broca, amygdala	Hypothalamo-hypophyseal tract, septum, thalamus, hippocampus	Neurohypophysis, median eminence OVLT, dorsomedial nucleus	+

+ = yes, in concentrations greater than peripheral blood.

is associated with OT, and NP-II or nicotine-linked neurophysin is associated with AVP. Both NPs are 9.5 to 10 kDa. The translation product of the AVP gene is a 21-kDa glycoprotein known as *proprovapressin* (Fig. 11) which, in the human, consists of a 19 amino acid (aa) signal peptide at the amino terminal, vasopressin (9 aa), a 3-aa spacer sequence, NP-II (93 aa), another spacer sequence (1 aa), and a 39-aa peptide at the carboxyl terminal. *Provapressin* (19 kDa) is the peptide with the 19-aa signal sequence cleaved and carbohydrate added posttranslationally to the C-terminal peptide. Oxytocin is synthesized in a similar fashion with the exception that there is no glycopeptide at the carboxyl terminus and the prohormone is

significantly smaller at 15 kDa (Fig. 11). In both cases, the NP is required as a carrier protein to transport the active peptide to the axon terminal by axoplasmic flow. During transport down the axon, the respective NP is cleaved from either OT or AVP by a family of membrane-associated aminopeptidase enzymes to release the active hormones, and the vesicles stored within the nerve terminal contain free peptide. In response to action potentials generated in the axons of neurosecretory neurons, the peptide is exocytosed from the axon terminals in the posterior pituitary gland and then diffuses via fenestrated capillaries into the bloodstream. OT and AVP are synthesized in distinct neuronal populations, although both

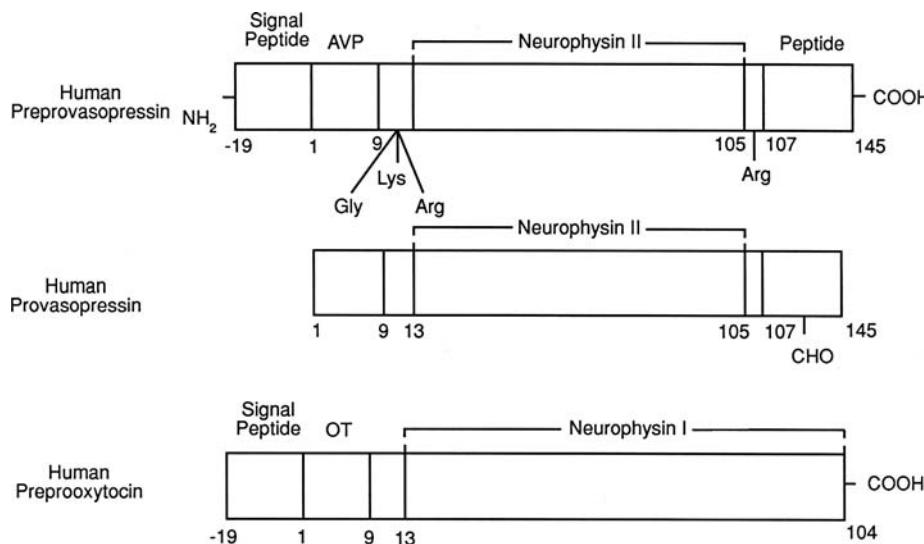


Fig. 11. The structure of human preprovasopressin, provasopressin, and preprotoxotocin. Note that preprovasopressin is 21 kDa and consists of a signal peptide, the AVP sequence, a tripeptide spacer, neurophysin II (also called nicotine-linked neurophysin), a spacer glycosylation signal (Arg), and a 39 amino acid carboxyl terminal. Provapressin is a 19-kDa product of preprovasopressin from which the signal sequence has been deleted and carbohydrate (CHO) added to the C-terminal peptide posttranslationally. Preprotoxotocin is smaller than preprovasopressin with a different neurophysin and the posttranslational modifications do not include glycosylation.

types of neuron are present in the supraoptic nucleus and the magnocellular region of the paraventricular nucleus. Axons from each of these nuclei form the *hypothalamo-hypophyseal tract*, which terminates upon sinusoids in the neurohypophysis. OT and AVP can also be transported from the neurohypophysis to the adenohypophysis through the short portal vessels connecting these two areas and thus may have a role in regulating anterior pituitary function.

OT and AVP are also produced by some neurons in the parvicellular portion (= small cell bodies, relative to magnocellular neurons) of the paraventricular nucleus. Axons from these neurons have a wide distribution in the brain. Some fibers project to the median eminence where they terminate upon the primary capillary plexus, which marks the start of the long portal vessels supplying blood to the adenohypophysis. Neuropeptides secreted here are transported directly to the anterior pituitary hormone and can regulate anterior pituitary function. This is the pathway by which AVP reaches the corticotroph and stimulates ACTH secretion. It is well-known that AVP is an *accessory ACTH-releasing factor* of hypothalamic origin. Similarly, oxytocin is secreted in this manner. Significant amounts of oxytocin are found in the pituitary portal blood, where it may play an important stimulatory role in the secretion of prolactin and luteinizing hormone from the lactotrophs and gonadotrophs, respectively. As well as projecting to the median eminence, parvicellular OT and AVP neurons also project to other parts of the central nervous system, including the septum, the locus coeruleus (LC), the amygdala, the parabrachial nuclei, the dorsal motor vagal nucleus, the nucleus of the solitary tract, the midbrain central gray, and the dorsal horn of the spinal cord. Localization of AVP and OT fibers in these regions reflects

the wide range of autonomic functions reported to be influenced by these neuropeptides, ranging from thermoregulation and blood pressure regulation through to complex traits such as social recognition and pair bonding, maternal behavior, learning, and memory.

Outside the supraoptic and paraventricular nucleus, AVP is also found in parvicellular neurons of the suprachiasmatic nucleus. These neurons project to the OVLT, the dorsomedial hypothalamic nucleus, and the thalamus. AVP-containing neurons have also been found in the bed nucleus of the stria terminalis, the nucleus of the diagonal band of Broca and lateral septum, the anterior amygdala, the lateral habenular nucleus, the mesencephalic central gray, and the locus coeruleus.

2.5.3. HYPOPHYSIOTROPIC PEPTIDES

The hormones that control the functions of the anterior pituitary gland (hypophysis) are also produced by parvicellular neurons scattered throughout various nuclei of the hypothalamus (Fig. 12; Table 6). These neurosecretory neurons project axons to the external layer of the median eminence, where their nerve terminals are found in close proximity to the fenestrated capillaries of the portal vasculature. The hypophysiotropic hormones are synthesized in the cell bodies of the neurons and are transported to and stored in the nerve terminals in the median eminence. The hormones are released by exocytosis, in response to action potentials propagated down the axons of the neurosecretory neurons. The relatively small size of the parvicellular neurons reflects the fact that these neurons are only required to produce relatively low levels of hormone. Secretions in the portal vasculature are transported directly to the anterior pituitary, undiluted by systemic blood. Hence, although relatively low levels are produced, these

GnRH: p¹Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly¹⁰-NH₂

TRH: p¹Glu-His-Pro³-NH₂

CRH: ¹Ser-Gln-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-Met
(ovine) Thr-Lys-Ala-Asp-Gln-Leu-Ala-Gln-Gln-Ala-His-Ser-Asn-Arg-Lys-Leu-Leu-Asp-Ile-Ala⁴¹NH₂

GHRH: ¹Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-
Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-
Arg-Leu⁴⁴-NH₂

Somatostatin: ¹Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys¹⁴

Fig. 12. Amino acid sequences of the *hypothalamic hypophysiotropic peptides*, so named because they all regulate pituitary function directly. The superscript “1” represents the amino terminus, whereas the carboxyl terminus is designated with the greater superscript number. Note that the Glu at position 1 of LHRH and TRH is designated pyro (p) and that LHRH, TRH, CRH, and GHRH all are amidated at their carboxy termini.

Table 6
Localization of the Hypophysiotropic Peptides

Peptide	Cell bodies	Fibers	Terminals	Presence in portal blood
GnRH	Medial septal nucleus, nucleus of the diagonal band of Broca, bed nucleus region of stria terminalis, OVLT, medial preoptic nucleus, anterior hypothalamic area, arcuate nucleus, median eminence, olfactory tubercle	Continuum from septal region to premammillary nucleus	Median eminence, neurohypophysis, suprachiasmatic nucleus, ependymal lining of ventricles, olfactory bulb, amygdala	+
TRH	Periventricular area, paraventricular nucleus, dorsomedial/ventromedial nuclei, arcuate nucleus	Paraventricular nucleus, periventricular hypothalamic area, dorsomedial nucleus, perifornical region, nucleus accumbens, bed nucleus of stria terminalis, spinal cord	Median eminence	+
CRH	Paraventricular nucleus, supraoptic nuclei, arcuate nuclei dorsal raphe nucleus, hippocampus, OVLT, medial preoptic nucleus, bed nucleus of stria terminalis, locus coeruleus	Septal nuclei, stria terminalis, median forebrain bundle	Median eminence	+
GHRH	Arcuate nucleus		Median eminence	+
SS	Preoptic/anterior hypothalamic area, paraventricular nucleus		Median eminence, suprachiasmatic nucleus	+

+ = yes, in concentrations greater than peripheral blood.

hormones are present in high concentrations because the volume of blood in the portal circulation is low.

2.5.3.1. Gonadotropin-Releasing Hormone. Of all the neuropeptides that control the function of the adenohypophysis, the chemiarchitecture of *gonadotropin-releasing hormone* (*GnRH*; also known as *luteinizing hormone–releasing hormone*, *LHRH*) is perhaps the most widely studied. GnRH-positive cell bodies fibers and terminals are not restricted to the hypothalamus. They are scattered over a continuum extending from the septal region to the premammillary region, although there is a great deal of variability in this distribution between different species (Fig. 13). GnRH neurons are predominately found in the septo-preoptic region, including medial septal nucleus, the nucleus of the diagonal band of

Broca at the level of the OVLT, the bed nucleus of the stria terminalis, the medial preoptic nucleus, and the anterior hypothalamic area. In humans and other primates, they are also found in the mediobasal hypothalamus, in the arcuate, infundibular, and premammillary nuclei. From all of these areas, GnRH neurons produce axons that pass to the infundibulum and form a dense plexus around the primary capillary bed of the hypothalamo-hypophyseal portal system in the median eminence. GnRH release from these neurons controls the secretion of LH and FSH from the adenohypophysis. GnRH-positive cells originating in the medial preoptic nucleus, the bed nucleus of the stria terminalis, and the septum also send fibers that terminate on the ependymal linings of the third and lateral ventricles. This suggests that the cerebrospinal fluid may be an additional vehicle for transport of GnRH. Axons from other GnRH-positive

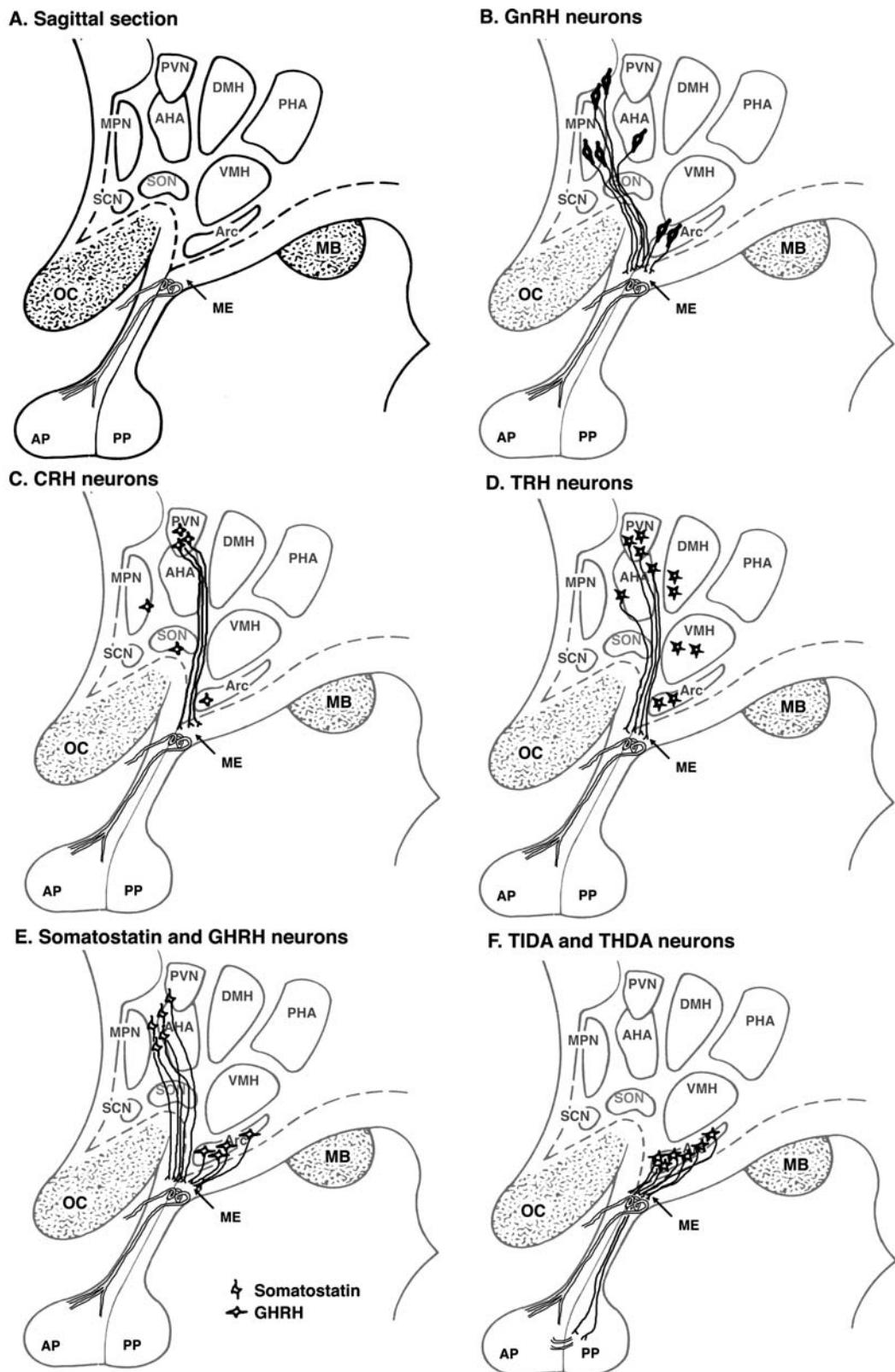


Fig. 13. (Continued)

cells in these areas terminate in the OVLT to form a dense plexus around the capillary network suggesting another vascular route through which the peptide reaches the circulation. The function of the OVLT projection is unknown. In addition, GnRH-positive projections from the medial preoptic nucleus, medial septal nucleus, and the diagonal band of Broca terminate in the external plexiform and glomerular layers of the olfactory bulb. The medial preoptic nucleus also provides GnRH fibers to the amygdala by way of the stria terminalis. Though the GnRH neurons originating in the preoptic and arcuate nuclei that terminate in the median eminence have been shown to play a direct role in cyclic LH release, termini in other areas such as the olfactory system, the amygdala, the habenula, and the mesencephalic gray have not been assigned a firmly established functional role in reproductive processes. However, it is probable that these areas play a role in behaviors related to reproduction that are controlled by GnRH not as a neurohormone but as a neuromodulator. Finally, it is interesting to note that GnRH is not confined to the central nervous system. In sympathetic ganglia of the bullfrog, a peptide that resembles GnRH and is colocalized with acetylcholine elicits prolonged excitatory postsynaptic potentials with long latencies. This would categorize GnRH as a neurotransmitter.

2.5.3.2. Thyrotropin-Releasing Hormone. Thyrotropin-releasing hormone (TRH) is the physiologic stimulator of TSH release from the adenohypophysis and is found widely throughout the nervous system of mammals. In fact, only approximately one-third of the total amount of the peptide found in the brain is localized to the hypothalamus. Thus, TRH is thought to play both a neuromodulatory and neuroendocrine

role. Extrahypothalamic structures such as the preoptic and septal areas as well as the motor nuclei of some cranial nerves also contain significant levels of TRH. Within the hypothalamus, TRH-positive cell bodies are found in the periventricular area, the paraventricular nucleus, the dorsomedial and ventromedial nuclei, and the arcuate nucleus/median eminence area (Fig. 13). TRH-positive nerve terminals are found in the greatest abundance in the external layer of the median eminence with dense fiber networks in the parvicellular part of the paraventricular nucleus, the periventricular hypothalamic area, the dorsomedial nucleus, the perifornical region, the nucleus accumbens, and the bed nucleus of the stria terminalis. The highest concentration of TRH in the hypothalamus is found in the median eminence with significant levels in the dorsomedial, ventromedial, and arcuate nuclei. TRH-positive fibers are also found in the spinal cord.

2.5.3.3. Corticotropin-Releasing Hormone. Corticotropin-releasing hormone (CRH) is the physiologic stimulator for the release of ACTH from the adenohypophysis. Like other hypophysiotropic hormones, however, it has been localized in both hypothalamic and extrahypothalamic sites. CRH-positive cells are found in greatest abundance in the paraventricular nucleus of the hypothalamus. Though most of the CRH neurons are parvicellular, CRH is also found in some of the magnocellular neurons of this nucleus. Axons from the CRH-positive cells of the paraventricular nucleus project to the external zone of the median eminence (Fig. 13), and it is these neurons that provide the CRH to the portal vasculature bathing the adenohypophysis and stimulating the release of ACTH and β -endorphin. In

Fig. 13. (Continued)

Diagrammatic representations of the distribution of neurons that secrete hypophysiotropic hormones. Note that all neurons project to the median eminence, where their nerve terminals are located in close proximity to the fenestrated capillaries of the primary capillary plexus of the hypophyseal portal system. (A) Diagram of sagittal section of the hypothalamus, showing the major nuclei (MPN, medial preoptic nucleus; PVN, paraventricular nucleus; AHA, anterior hypothalamic area; DMH, dorsomedial hypothalamic nucleus; PHA, posterior hypothalamic area; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; VMH, ventromedial hypothalamic nucleus; Arc or Arn, arcuate nucleus; OC, optic chiasm; ME, median eminence; MB, mammillary bodies; AP, anterior pituitary; PP, posterior pituitary. (B) Distribution of GnRH neurons. In addition to localization in the MPN, AHA, and Arc (shown), cell bodies are found more rostrally in the septum and diagonal band of Broca at the level of the OVLT. (C) Distribution of CRH neurons. Major hypophysiotropic projection from the PVN is shown. Cell bodies are also located in the MPN, SON, and Arc, but these may project elsewhere. (D) Distribution of TRH neurons. Again, the major hypophysiotropic projection from the PVN is shown. Cell bodies also located in the DMH, VMH, and Arc. (E) Distribution of somatostatin and GHRH neurons. Many somatostatin cell bodies are located in the periventricular nucleus (medial to the AHA, not shown). (F) Distribution of the hypophysiotropic dopamine neurons, located in the arcuate nucleus. The tuberoinfundibular dopaminergic (TIDA) neurons project to the median eminence, whereas the tuberohypophysial dopaminergic (THDA) neurons project to the posterior pituitary. Dopamine from THDA neurons may reach the anterior pituitary via short portal vessels.

addition, scattered CRH-positive cell bodies have been identified throughout the lateral preoptic-lateral hypothalamic continuum, in the medial preoptic nucleus and the bed nucleus of the stria terminalis, and also in the supraoptic and arcuate nuclei in the hypothalamus. Additional groups of cell bodies have been detected in the dorsal raphe nucleus, the amygdala, the hippocampus, the midbrain, the reticular formation, and the periaqueductal gray. Moreover, CRH has been colocalized with the catecholamines in the locus coeruleus. CRH-immunoreactive fibers originating from the bed nucleus of the stria terminalis enter the lateral and medial septal nuclei. Numerous fibers found within the stria terminalis and the ventral amygdalofugal pathway connect the rostral hypothalamus and basal telencephalon with the amygdala. Fibers originating from the telencephalon/diencephalon course caudally through the median forebrain bundle and split into a dorsal pathway throughout the brain stem and a ventral pathway to the lateral part of the reticular formation. Though a well-founded physiologic role has been ascribed to the hypothalamic paraventricular-median eminence pathway, a role for the other pathways has not been similarly characterized. It is possible that CRH in limbic regions such as the amygdala might be involved in anxiety-related pathways.

2.5.3.4. Growth Hormone-Releasing Hormone.

Growth hormone-releasing hormone (GHRH) is the physiologic stimulator of growth hormone secretion from the somatotrophs of the adenohypophysis, operating in concert with somatostatin (see later) to generate a pulsatile pattern of growth hormone secretion. Immunocytochemical localization studies have revealed GHRH-positive cell bodies in the arcuate nucleus with short axons terminating in the arcuate nucleus and the median eminence (Fig. 13). The concentration of immunoreactive GHRH in the hypothalamus is highest in the median eminence, reflecting its key neuroendocrine role at the pituitary gland. Some GHRH cell bodies coexpress the neuropeptide neurotensin, whereas others contain galanin. The physiologic significance of this dual packaging is unknown. Interestingly, surgical isolation of the medial basal hypothalamus does not lead to a significant decline in the concentration of GHRH in the arcuate nucleus median eminence area. Thus, virtually all of the hypothalamic GHRH originates from cells in this area. There are also significant amounts of GHRH found in some non-neural sites. Specifically, both GHRH messenger RNA and newly synthesized GHRH are found

in somatotrophs of the adenohypophysis. This has led to the belief that some degree of growth hormone secretion is intrinsic through an autocrine relationship. Other locations of GHRH cells lack a compelling physiologic explanation. GHRH has been found in the ovary and the placenta; sites at which a role for GHRH has yet to be described.

2.5.3.5. Growth Hormone Release-Inhibiting Hormone, or Somatostatin.

As its name implies, somatostatin inhibits the secretion of growth hormone or somatotropin from the adenohypophysis. However, the name does not fully represent the variety of roles played by this neurohormone. Somatostatin also inhibits the release of thyrotropin and prolactin from the adenohypophysis. In addition to its location in the hypothalamus, somatostatin is widely distributed throughout the central nervous system suggesting that it may be a neurotransmitter or neuromodulator as well as a neurohormone. Of interest to control of growth hormone secretion, somatostatin-positive cell bodies are abundant in the periventricular preoptic-anterior hypothalamic area. In addition, parvicellular somatostatin-positive cells are also found in the paraventricular nucleus. These areas send axons as a group caudally to terminate in the suprachiasmatic nucleus as well as the arcuate nucleus/median eminence area (Fig. 13). Fibers passing from the periventricular preoptic area terminate on the primary capillary bed of the median eminence. This is the source of somatostatin that directly inhibits growth hormone secretion from the adenohypophysis. Moreover, these same preoptic somatostatin fibers synapse on GHRH cell bodies in the arcuate nucleus. This suggests two levels of inhibition of growth hormone secretion by somatostatin: directly at the somatotroph and secondarily at the GHRH neuron. Finally, somatostatin can be found outside of the nervous system. Within the endocrine pancreas a specific cell type, the delta cell, synthesizes and secretes somatostatin that is identical to that made by hypothalamic neurons. Pancreatic somatostatin plays numerous roles in the gastrointestinal tract. In addition, somatostatin directly affects pancreatic insulin and glucagon secretion.

2.5.3.6. Prolactin Inhibitory Factor: Dopamine.

Though not peptide-producing neurons, the neuroendocrine dopamine neurons of the hypothalamus must be considered in this section, as they form the major regulatory mechanism controlling prolactin secretion from the adenohypophysis. Unlike all other pituitary

hormones, prolactin secretion is predominately inhibited by the hypothalamus by means of an inhibitory hormone. Much research has now confirmed that this inhibitory hormone is dopamine, a catecholamine released from neuroendocrine neurons in the basal hypothalamus. The cell bodies of the *tuberoinfundibular dopamine neurons* are located in the arcuate nucleus with short axons that terminate in the median eminence (Fig. 13). These converge upon the primary capillary bed of the hypophyseal portal system and thus have been shown to play a direct role in the release of prolactin from the adenohypophysis. The *tuberohypophyseal dopamine neurons* have cell bodies in the rostral arcuate and periventricular nuclei with axons terminating in the intermediate and posterior lobes of the pituitary gland. In the neurohypophysis, these axons lie in close proximity to vascular spaces, neurosecretory axons, and pituicytes (modified astroglial cells). Within the intermediate lobe, these axons terminate on secretory cells known as melanotrophs. It is believed that a portion of the dopamine acting within the adenohypophysis originates from the axon terminals in the intermediate and posterior lobes and ultimately reaches the anterior pituitary by means of short portal vessels.

2.5.4. OTHER HYPOTHALAMIC PEPTIDES

Seven arbitrary classes of peptides are involved in hypothalamic function (Table 3): The neurosecretory *neurohypophyseal peptide* and *hypophysiotropic peptides* have been described above. In addition, there are a range of neuropeptides that are secreted from nerve terminals within the hypothalamus and involved in neuromodulatory role in hypothalamic

function: the *proopiomelanocortin (POMC)-derived peptides* (Fig. 14), including α -melanocyte stimulating hormone and β -endorphin; the *opioid peptides* enkephalin and dynorphin (Fig. 15); the *brain-gut peptides* (Fig. 16) including vasoactive intestinal peptide (VIP), cholecystokinin (CCK), substance P, and neuropeptidene. Recently, a new class of peptides, the *RF-amides*, including kisspeptin and prolactin-releasing peptide, have been identified in the hypothalamus (Fig. 17) and have important roles in regulating hypothalamic function. Finally, *other peptides* such as angiotensin II, neuropeptide y, orexin, galanin, and the endothelins (Fig. 18) could stand as a class by themselves as they may play a variety of neuroendocrine, neuromodulatory, neurotransmitter, and hormonal roles. The following is a description of the peptides that are found in the hypothalamus as well as those found outside of the hypothalamus but effect hypothalamic function.

2.5.4.1. POMC-Derived Peptides. *POMC* is a large-molecular-weight precursor protein (265 aa) that in the human (with subtle differences between mammals) is posttranslationally cleaved into moieties *ACTH* (39 aa), β -*lipotropin* (β -*LPH*; 89 aa), and a 16-Kd *N-terminal fragment* of 76 aa (Fig. 19). Each of these is further cleaved enzymatically to yield additional bioactive peptides. *ACTH* is cleaved into α -*melanocyte stimulating hormone* (α -*MSH* = *ACTH*₁₋₁₃) and *corticotropin-like intermediate lobe peptide* (*CLIP* = *ACTH*₁₈₋₃₉). β -*LPH* is further cleaved into γ -*LPH* (β -*LPH*₁₋₅₆) and the endogenous opioid β -*endorphin* (β -*END* = β -*LPH*₅₉₋₈₉). β -*MSH*

ACTH: ¹Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Leu-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe³⁹

β -LPH: ¹Glu-Leu-Thr-Gly-Gln-Arg-Leu-Arg-Glu-Gly-Asp-Gly-Pro-Asp-Gly-Pro-Ala-Asp-Asp-Gly-Ala-Gly-Ala-Gln-Ala-Asp-Leu-Glu-His-Ser-Leu-Leu-Val-Ala-Ala-Glu-Lys-Lys-Asp-Glu-Gly-Pro-Tyr-Arg-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp-Lys-Arg-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly⁸⁹

α -MSH: Ac-¹Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val¹³-NH₂

α -LPH: ¹Glu-Leu-Thr-Gly-Gln-Arg-Leu-Arg-Glu-Gly-Asp-Gly-Pro-Asp-Gly-Pro-Ala-Asp-Asp-Gly-Ala-Gly-Ala-Gln-Ala-Asp-Leu-Glu-His-Ser-Leu-Leu-Val-Ala-Ala-Glu-Lys-Lys-Asp-Glu-Gly-Pro-Tyr-Arg-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp⁵⁶

β -END: ¹Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly³¹

β -MSH: ¹Asp-Glu-Gly-Pro-Tyr-Arg-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp¹⁸

α -MSH: ¹Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-Gly¹²

Fig. 14. Amino acid sequences of the hypothalamic peptides derived from *proopiomelanocortin*.

Met-Enk: $^1\text{Tyr-Gly-Gly-Phe-Met}^5$

Leu-Enk: $^1\text{Tyr-Gly-Gly-Phe-Leu}^5$

Dynorphin: $^1\text{Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln}^{17}$

Fig. 15. Amino acid sequences of the opioid peptides dynorphin and the *enkephalins*. Note that the sole difference between the two enkephalin molecules is in position 5.

is a cleavage product of LPH (39-56) and γ -MSH is a fragment of the 16-K N-terminal peptide. These are widely distributed in the central nervous system (Table 7).

Aside from the adenohypophysis, ACTH is found in cells of the arcuate nucleus/median eminence area. The cells are diffusely distributed throughout this area, which extends rostrally to the retrochiasmatic area, caudally to the submammillary region, and dorsally to the area between the ventricular surface and the ventromedial nucleus of the hypothalamus. That this activity is *not* a product of the corticotrophs of the adenohypophysis is emphasized by the fact that hypophysectomy does not influence the amount of ACTH in this area. Thus, ACTH is actually formed in the brain but may serve as a precursor to other bioactive peptides, particularly α -MSH. Because ACTH and other peptides are common products of POMC, it is not surprising that β -LPH, α -MSH, β -MSH and β -END are colocalized with ACTH in these neurons. These neurons give rise to numerous ACTH-positive fibers that are distributed widely throughout the brain. Within the hypothalamus, ACTH fibers terminate in the anterior, mediobasal, and periventricular areas of the hypothalamus. In the periventricular area the fibers actually penetrate the ependymal lining of the third ventricle. ACTH is measurable in the cerebral spinal fluid. ACTH terminals are found in the dorsomedial nucleus, the

magnocellular and parvicellular portions of the paraventricular nucleus, and the OVLT. In addition, ACTH terminals are found in the external zone of the median eminence close to the portal capillaries as well as within the neurohypophysis. Terminals are also found in the medial preoptic area.

β -LPH cell bodies are also found in the arcuate nucleus/median eminence area with fibers projecting to various areas of the brain. In general, ACTH-positive fibers and β -LPH fibers project to the same areas of the brain. β -LPH is also found in the corticotrophs of the adenohypophysis. This common distribution patterns are not too surprising given the common precursor of both.

α -MSH is secreted from the intermediate lobe of the pituitary gland in all vertebrates. Though it has a dramatic skin coloring effect in poikilotherms, a role for α -MSH in homeotherms is uncertain. There is some suggestion that it may play a role in secretion of hormone from the adenohypophysis. Within the hypothalamus, α -MSH-positive cell bodies and fibers are found in the same areas of the arcuate nucleus/median eminence that were described for ACTH, and α -MSH from these neurons plays a major role in the suppression of appetite, described in Section 4.3.1.4. The fibers, for the most part, project to the same areas as the ACTH-positive groups. There is also a second group of α -MSH-positive cells that are distinct from those in the medial basal hypothalamus. These cells are concentrated in the area between the dorsomedial nucleus and in the fornix and in the lateral hypothalamic area. These cells do not colocalize α -MSH with any other of the POMC-derived peptides suggesting that the biosynthetic route of α -MSH in these cells might be quite different than that of the mediobasal hypothalamus or the intermediate lobe of the pituitary gland. Fibers from this group project to the caudate-putamen complex, the neocortex, and various parts of the hippocampus.

Substance P: $^1\text{Arg-Pro-Lys-Pro-Glu-Glu-Phe-Phe-Gly-Leu-Met}^{11}\text{-NH}_2$

VIP: $^1\text{His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Glu-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn}^2\text{-NH}_2$

CCK (8): $^1\text{Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe}^8\text{-NH}_2$
 $\begin{array}{c} | \\ \text{SO}_3 \end{array}$

NT: $p^1\text{Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu}^{13}$

Fig. 16. Amino acid sequences of the hypothalamic *brain-gut peptides*, so named because they are localized and active in both the brain and gastrointestinal system.

Kisspeptin:	¹ Glu - Thr - Ser - Leu - Ser - Pro - Pro - Pro - Glu - Ser - Ser - Gly - Ser - Arg - Gln - Gln - Pro - Gly - Leu - Ser - Ala - Pro - His - Ser - Arg - Gln - Ile - Pro - Ala - Pro - Gln - Gly - Ala - Val - Leu - Val - Gln - Arg - Glu - Lys - Asp - Leu - Pro - Asn - Tyr - Asn - Trp - Asn - Ser - Phe - Gly - Leu - Arg - Phe ⁵⁴ - NH ₂
PrRP:	¹ Ser - Arg - Thr - His - Arg - His - Ser - Met - Glu - Ile - Arg - Thr - Pro - Asp - Ile - Asn - Pro - Ala - Trp - Tyr - Ala - Ser - Arg - Gly - Ile - Arg - Pro - Val - Gly - Arg - Phe ³¹ - NH ₂
GnIH (RFRP - 3):	¹ Ala - Gly - Ala - Thr - Ala - Asn - Leu - Pro - Leu - Arg - Ser - Gly - Arg - Asn - Met - Glu - Val - Ser - Leu - Val - Arg - Arg - Val - Pro - Asn - Leu - Pro - Gln - Arg - Phe ³⁰ - NH ₂

Fig. 17. Amino acid sequences of representative RF-amide peptides expressed in the hypothalamus, so-called because they share an arginine (R) phenylalanine (F) amide at the carboxy terminal.

Angiotensin II:	¹ Asp-Arg-Val-Tyr-Ile-His-Pro-Phe ⁸
Human NPY:	¹ Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Gln-Asp-Ala-Pro-Ala-Gln-Asp-Met-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr ³⁶ -NH ₂
Rat Galanin:	¹ Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-Leu-Gly-Pro-His-Ala-Ile-Asp-Asn-His-Ag-Ser-Phe-Ser-Asp-Lys-His-Gly-Leu-Thr ²⁹ -NH ₂
Endothelin 1:	¹ Cys-Ser-Cys-Ser-Ser-Leu-Met-Asp-Lys-Glu-Cys-Val-Tyr-Phe-Cys-His-Leu-Asp-Ile-Ile-Trp ²¹
Endothelin 2:	¹ Cys-Ser-Cys-Asp-Ser-Trp-Leu-Asp-Lys-Glu-Cys-Val-Tyr-Phe-Cys-His-Ile-Ile-Trp ²¹
Endothelin 3:	¹ Cys-Thr-Cys-Phe-Thr-Tyr-Lys-Asp-Lys-Glu-Cys-Val-Tyr-Tyr-Cys-His-Leu-Asp-Ile-Ile-Trp ²¹
Orexin A:	¹ Gln-Pro-Leu-Pro-Asp-Cys-Cys-Arg-Gln-Lys-Thr-Cys-Ser-Cys-Arg-Leu-Tyr-Glu-Leu-Leu-His-Gly-Ala-Gly-Asn-His-Ala-Ala-Gly-Ile-Leu-Thr-Leu ³³

Fig. 18. Amino acid sequences of some of the *other peptides* localized in the hypothalamus and controlling hypothalamic function. Among the unusual features, note that the endothelins have two intramolecular disulfide bonds.

As with the above POMC-derived peptides, β -END-positive cell bodies are most numerous in the arcuate nucleus/median eminence area of the hypothalamus, and the course of their efferent projections is similar to ACTH, LPH, and MSH. Similarly, β -END is found in the intermediate lobe of the pituitary gland as well. β -END is also found in significant concentrations in hypophyseal portal blood. Thus β -END qualifies as a neurotransmitter/neuromodulator as well as a neurohormone. Because β -END binds to opiate receptors throughout the nervous system, it has been classified an endogenous opioid.

2.5.4.2. Opioid Peptides. The opioid peptides are derived from three different precursors. The derivation of β -END from POMC was described above.

Smaller opioids, the enkephalins (ENK), are pentapeptides derived from larger molecules known as *proenkephalins* (Fig. 20). One, proenkephalin A (50 kDa, 267 aa), contains four copies of *methionine-ENK* (*met-ENK*) and one copy of *leucine-ENK* (*leu-ENK*), and one copy each of a met-ENK C-terminal heptapeptide and a met-ENK C-terminal octapeptide. The other, proenkephalin B (also known as prodynorphin), contains three copies of leu-ENK, the latter two of which form the first five amino acids of additional opioid peptides, dynorphin A and dynorphin B. The most widely distributed opioid peptides are the enkephalins with met- and leu-ENK found in the same areas. In general, met-ENK is found in higher concentrations than is leu-ENK. With regard to the hypothalamus (Table 8),

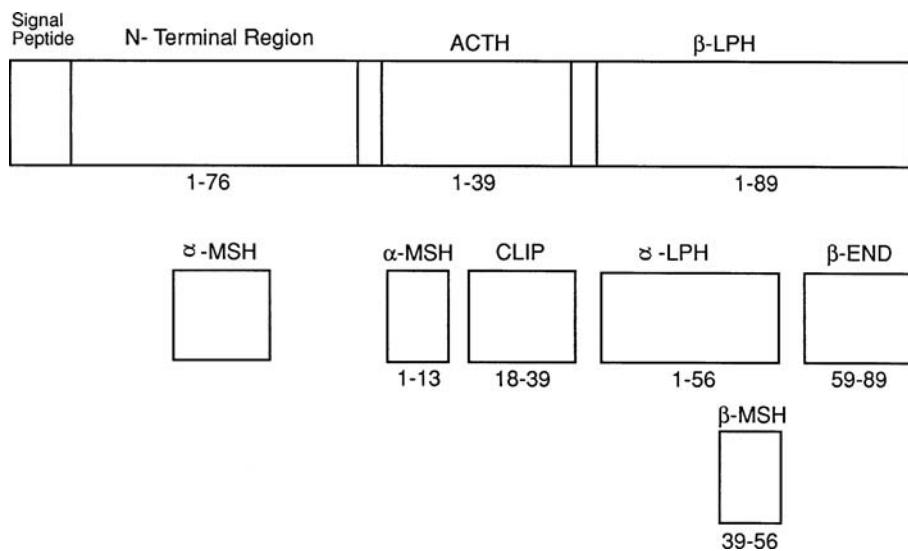


Fig. 19. Human proopiomelanocortin and its cleavage products. The numbers below each bar represent the number of amino acids of the parent peptides (*upper bar*) or the position in the parent peptides from which the products are cleaved (*middle and lower bars*).

ENK-positive cell bodies are found in the supraoptic and paraventricular nuclei. ENK-positive efferents project from these areas and terminate in the external zone of the median eminence adjacent to the portal vessels and in the neurohypophysis. The medial preoptic nucleus as well as the dorsomedial and ventromedial hypothalamic nuclei also contain ENK-positive cell bodies. Met-ENK is also coexpressed in tuberoinfundibular dopamine neurons and is reported to be present in portal blood. In addition, cells in the intermediate lobe of the pituitary gland contain the hepta- and octapeptide of met ENK but

not free met-ENK. In the adenohypophysis, gonadotrophs contain all forms of met-ENK. These are not the same gonadotrophs that colocalize β-END. Moreover, there is a population of gonadotrophs that also contain prodynorphin. The ENKs appear to regulate pituitary hormone secretion by acting as neuromodulators/neurotransmitters, regulating secretion of other hypothalamic peptides. In the neurohypophysis, ENK inhibits the release of OT and AVP. In the adenohypophysis, ENK inhibits LH and stimulates PRL, growth hormone, and ACTH secretion by acting within the hypothalamus as a

Table 7
Localization of POMC-Derived Peptides

Peptide	Cell bodies	Terminals	Presence in portal blood
ACTH	Arcuate nucleus, median eminence area	Anterior hypothalamic periventricular area, dorsomedial nucleus, paraventricular nucleus, OVLT, median eminence, preoptic area	+ *
β-LPH	Same as ACTH		ND
γ-MSH	Same as ACTH		+ *
β-END	Same as ACTH		+

+ = yes, in concentrations greater than peripheral blood.

* = probably by retrograde blood flow from pituitary gland.

ND = not determined.

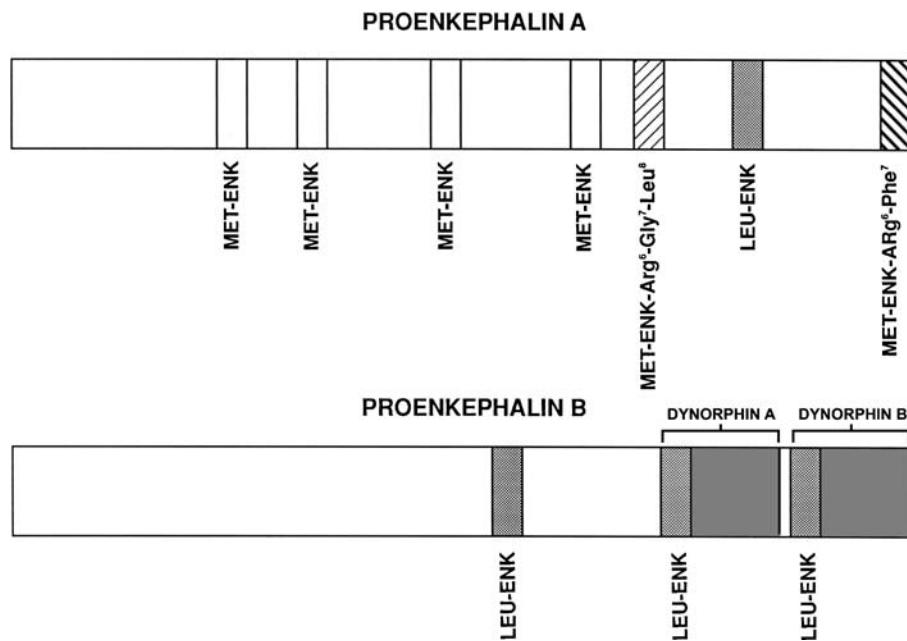


Fig. 20. The structure of proenkephalin A and proenkephalin B (prodynorphin). Note the 4 repeating met-ENK sequences, the single leu-ENK, and each single met-ENK heptapeptide and octapeptide characteristic of proenkephalin A. Proenkephalin B is characterized by 3 intramolecular leu-ENK sequences. The second and third Leu-Enk represent the first five amino acids of dynorphin A and dynorphin B, respectively.

neuromodulator/neurotransmitter. The colocalization of the ENKs with pituitary hormones suggests a paracrine or autocrine role of ENK but the only direct effect has been shown in the neurohypophysis on the inhibition of OT and AVP.

Dynorphin expression is widespread in the brain and spinal cord. Within the hypothalamus, it has been identified in the supraoptic nucleus and paraventricular nucleus, where it appears to be coexpressed with AVP. Dendritic release of dynorphin

Table 8
Localization of Enkephalins

Peptide	Cell bodies	Terminals	Presence in portal blood
Met-Enk	Supraoptic nucleus, Paraventricular nucleus, preoptic area, dorsomedial/ventromedial nuclei	Median eminence, neurohypophysis	+
Leu-Enk	Same as MET-ENK		+
Dynorphin	Supraoptic nucleus, paraventricular nucleus, preoptic area, anterior hypothalamus area, bed nucleus of the stria terminalis, ventromedial nucleus (VMN), dorsomedial nucleus of the hypothalamus, and the arcuate nucleus	Extensive throughout brain, including striatum, hippocampus, brain stem, and spinal cord. Within hypothalamus, preoptic area, anterior hypothalamus, paraventricular nucleus, supraoptic nucleus, arcuate nucleus	?

+ = yes, in concentrations greater than peripheral blood.

? = unknown.

from AVP neurons has been shown to be important in regulating the firing pattern of these neurons in an autocrine manner. It is also found in the preoptic area, anterior hypothalamus area, bed nucleus of the stria terminalis, ventromedial nucleus (VMN), dorsomedial nucleus of the hypothalamus, and the arcuate nucleus. In the arcuate nucleus, it appears to be coexpressed with neurokinin B and kisspeptin.

2.5.4.3. Brain-Gut Peptides. This group of peptides is so-named because they were originally identified within the gastrointestinal tract and subsequently identified within the hypothalamus (Table 9). In addition to many of them being localized in the hypothalamus, a neuroendocrine role for some have been identified.

Substance P (SP) has been found in extracts of brain and intestine. Its structure is known, and it has been implicated in pain perception, baroreception, and chemoreception. In the monkey, SP cell bodies have been found in the most lateral portions of the arcuate nucleus. Fibers pass to the external zone of the median eminence and also to the neurohypophysis. In the rat, SP-positive cells are found in the medial and lateral preoptic areas, the anterior hypothalamic area, and the dorsomedial and ventromedial nuclei. SP-containing afferent pathways project to the supraoptic and paraventricular nuclei as well as the arcuate nucleus. However, in spite of the location of

SP fibers in the neurohemal zone of the median eminence, the levels of SP in portal blood is essentially equivalent to that of peripheral blood; thus eliminating it as a neurohormone. SP and its receptors are also found within the adenohypophysis, but SP may function as a paracrine agent rather than as a neurohormone. SP plays a role in the secretion of all the important anterior pituitary hormones either by acting directly on the specific cells of the gland or indirectly as a neuromodulator affecting the release of the releasing hormones of hypothalamic origin.

Vasoactive intestinal polypeptide (VIP) is present in large quantities throughout the gastrointestinal tract where it plays multiple roles in digestive processes. For example, it is vasodilatory, glycogenolytic, and lipolytic. It enhances insulin secretion, inhibits gastric acid production, and stimulates secretion from the exocrine pancreas and small intestine. Because it exerts some of its effects through vascular pathways, it fits the description of a true hormone. Neuronal VIP is found in highest concentration in the cerebral cortex where it acts as an excitatory neurotransmitter or neuromodulator. Within the hypothalamus, the suprachiasmatic nuclei contain very dense concentrations of VIP-positive cell bodies. Efferents from these course dorsally and then split into a dense rostro-dorsal component and a less dense caudal component and seem likely to play a role in relaying circadian information from the suprachiasmatic nucleus to target neurons. The

Table 9
Localization of Brain-Gut Peptides

Peptide	Cell bodies	Terminals	Presence in portal blood
Substance P	Arcuate nucleus, preoptic area, anterior hypothalamic area, dorsomedial/ventromedial nuclei	Median eminence, neurohypophysis, supraoptic nucleus, paraventricular nucleus, arcuate nucleus	+
VIP	Suprachiasmatic nucleus	Paraventricular nucleus dorsomedial/ventromedial nuclei	+
CCK	Cortex, striatum, amygdala supraoptic nucleus, paraventricular nucleus, neurohypophysis, preoptic area, dorsomedial nucleus	Median eminence	-
NT	Preoptic area, anterior hypothalamic area, medial preoptic area, paraventricular nucleus, dorsomedial nucleus, arcuate nucleus	Median eminence, neurohypophysis	+

+ = yes, in concentrations greater than peripheral blood.

- = no, concentrations the same as or lower than peripheral blood.

rostral fibers terminate on the paraventricular nucleus whereas the caudal fibers terminate at the dorsomedial, ventromedial, and premammillary nuclei. Like SP, VIP is found in high concentrations in the adenohypophysis. However, unlike SP it is also found in high concentrations in portal blood. There is also evidence that it may be synthesized in the adenohypophysis. VIP has been shown to affect adenohypophyseal hormone secretion as a transmitter/neuromodulator, as a neurohormone, and as a local autocrine or paracrine agent.

Cholecystokinin (CCK) is synthesized in the duodenum and stimulates the secretion of pancreatic enzymes and the ejection of bile from the gall bladder. CCK occurs in multiple molecular forms, which complicates the description of its tissue distribution. Duodenal CCK is composed of 33 or 39 amino acids. The carboxy terminal octapeptide of CCK (CCK8), which has full biological activity, shares a pentapeptide sequence with gastrin, another gastrointestinal hormone. However, for the most part, whereas CCK8 occurs throughout the central nervous system, gastrin-like peptides are found only in the pituitary gland and hypothalamus. Highest concentrations of immunoreactive CCK8 are found in the cortex, striatum, and amygdala with lesser amounts in the hypothalamus. CCK-like immunoreactivity has been found in the magnocellular systems of the supraoptic and paraventricular nuclei of the hypothalamus as well as the neurohypophysis. In some neurons, OT and CCK are colocalized. Physiologic perturbations that stimulate the release of AVP and OT lower neurohypophyseal CCK. Parvicellular CCK-positive cells project axons that terminate in the median eminence. In addition to these, CCK cells are found in the medial preoptic area as well as the dorsomedial and supramammillary nuclei. Functionally, CCK has been implicated as a neuromodulator in the control of pituitary hormone release. As such, it probably facilitates the release of the hypothalamic releasing hormones as well as OT and AVP. It has also been shown that CCK may be copackaged with many of the hypothalamic peptides. There is no solid direct evidence implicating CCK as a neurohormone.

Neurotensin (NT) is a peptide consisting of 13 amino acids that was first isolated from brain and later from the intestine. In general, NT is a peptide neurotransmitter that is found in highest concentrations in the hypothalamus and seems to play a role in the control of release of the adenohypophyseal hormones. NT-positive cell bodies are found in the

preoptic and anterior hypothalamic areas, the medial preoptic nucleus, the magnocellular and parvicellular zones of the paraventricular nucleus, the arcuate nucleus, and the dorsomedial nucleus. In the arcuate nucleus, NT is colocalized in tuberoinfundibular dopaminergic neurons. The biological significance of this common packaging is not as yet fully appreciated. There is, however, the suggestion that NT mediates the release of dopamine into the portal vasculature. NT-positive fibers are also localized to the external zone of the median eminence. In addition, NT is present in portal blood and thus may assume the role of a classic neurohormone. Though NT, placed into the brain, can affect the secretion of prolactin, growth hormone, TSH, and LH, placement directly into pituitary cell cultures is only effective at supraphysiologic doses. This suggests that NT acts within the hypothalamus as a neuromodulator/neurotransmitter and perhaps not at the pituitary cell directly. In addition to NT fibers terminating at the external zone of the median eminence, some NT-positive axon terminals are found in the neurohypophysis. These probably originate in the paraventricular nucleus and play a role in the release of OT and AVP. Finally, the adenohypophysis contains cells that stain NT-positive. It seems unlikely that this material arises from neuronal sources but probably is synthesized directly in the pituitary and plays a paracrine or autocrine role in the secretion of one or more of the adenohypophyseal hormones.

2.5.4.4. RF-Amides. This is a recently-identified family of peptides, so called because of the arginine (R) and phenylalanine (F) amino acids at their carboxy terminus. Because of their localized expression within the hypothalamus, they have generated a lot of excitement as potentially important regulators of hypothalamic function. Despite early promise, some of the enthusiasm that led to the naming of the peptides appears to have been misplaced. For example, *prolactin-releasing peptide (PrRP)*, localized in the caudal dorsomedial hypothalamic nucleus (and also in the brain stem), was originally identified as a specific stimulator of prolactin secretion from anterior pituitary cells. The neuroanatomy suggests, however, that PrRP neurons do not project to the median eminence or infundibulum, and hence, this peptide cannot reach the pituitary gland. A number of other functions have now been suggested, including regulation of food intake and energy balance. Similarly, *gonadotropin-inhibitory hormone (GnIH)*

was first identified in the bird hypothalamus, and in birds it seems to play a role in opposing the actions of GnRH to inhibit pituitary gonadotropin secretion. A mammalian homologue has now been identified, expressed in the dorsomedial hypothalamic nucleus, but it seems unlikely that the peptide is released into the pituitary portal blood. Hence, the function of this peptide in mammals is not known. Possibly the most exciting discovery of the past few years is the identification of the peptide *Kisspeptin* (also known as *metastin*), expressed in the arcuate nucleus and anteroventral periventricular nuclei of the hypothalamus. This peptide is exquisitely sensitive to gonadal steroid regulation, particularly estradiol. It profoundly stimulates GnRH neurons in the hypothalamus and may be a key player in the activation of these neurons at puberty. It also appears to be an essential part of the positive feedback mechanism by which rising levels of estradiol from the developing ovarian follicle induce a preovulatory GnRH and consequent LH surge, leading to ovulation.

2.5.4.5. Other Hypothalamic Peptides. A large range of other peptides have been described, and on the basis of their neuroanatomic location in the hypothalamus and/or pharmacologic studies, proposed as important regulators of hypothalamic function (Table 10). Though the evidence for their physiologic significance is incomplete, several of these peptides should be mentioned as potential physiologic regulators of hypothalamic function.

One peptide, *angiotensin II (AII)*, plays a role in vasoconstriction, sodium retention, antidiuresis, and drinking behavior through direct actions on peripheral structures such as the adrenal cortex and kidney, an action on the circumventricular organs such as the OVLT, the area postrema and the subfornical organ, and a direct action on the hypothalamus. There is also physiologic evidence that angiotensin II affects the secretion of LH and prolactin from the adenohypophysis through a neuromodulator/neurotransmitter, neurohumoral, and even a paracrine or autocrine role. AII is formed by the action of a renal proteolytic enzyme, *renin*, acting upon a peptide produced in the

Table 10
Localization of “Other” Hypothalamic Peptides

Peptide	Cell bodies	Terminals	Presence in portal blood
AII	Paraventricular nucleus, supraoptic nucleus, adenohypophysis	Median eminence, neurohypophysis, dorsomedial nucleus	-
NPY	Arcuate nucleus, median eminence, dorsomedial nucleus, locus coeruleus	Medial preoptic area, anterior hypothalamic area, periventricular area, suprachiasmatic, supraoptic, paraventricular, arcuate, and ventromedial nuclei, median eminence	+
Galanin	Supraoptic, paraventricular and arcuate nuclei	Median eminence, neurohypophysis	+
Orexin	Lateral hypothalamus	Throughout hypothalamus Extrahypothalamic projections throughout brain, with dense projection to locus coeruleus, and fibers in the septal nuclei, bed nucleus of the stria terminalis, thalamus, zona incerta, subthalamic nucleus, midbrain central gray, substantia nigra, raphe nuclei, parabrachial area, medullary reticular formation, and the nucleus of the solitary tract. Less prominent projections found in cortical regions, central and anterior amygdaloid nuclei, and olfactory bulb.	?

+ = yes, in concentrations greater than peripheral blood.

- = no, concentrations the same as or less than peripheral blood.

? = unknown.

liver, *angiotensinogen*, to form a circulating decapeptide, *angiotensin I* (AI). AI, in turn, is cleaved by an *angiotensin-converting enzyme (ACE)* produced in the lungs to form the biologically active octapeptide AII. Peripherally, AII acts on smooth muscle in arterial walls to promote vasoconstriction and raise blood pressure. Application of AII directly to the circumventricular organ also evokes an increase in blood pressure, secretion of AVP, and short-latency drinking behavior. The circumventricular organs are outside of the blood-brain barrier and possess a significant number of AII receptors. AII stimulates the adrenal cortex to secrete aldosterone, the hormone that promotes sodium retention by the nephron. There are certain hypothalamic and extra-hypothalamic structures that express AII receptors and are sensitive to the application of AII. The preoptic area contains a large number of AII receptors and its cells increase their firing rate when AII is applied microiontophoretically. These cells mediate the dipsogenic effects of AII. The paraventricular nucleus is also sensitive to AII. It is not clear how peripheral AII gains access to these centers. However, there is abundant evidence for the existence of AII-producing elements within the central nervous system. In fact, AII-producing cell bodies are found within the magnocellular cells of the paraventricular nucleus as well as within the supraoptic nucleus. The efferent projections of these cells terminate within the median eminence as well as within the neurohypophysis. AII terminals are also found concentrated in the dorsomedial nucleus of the hypothalamus and scattered throughout the medial basal hypothalamus. It has been reported that AII and AVP are copackaged and that AII, renin, and OT are also copackaged. In the adenohypophysis, AII has been reported to be packaged in gonadotrophs. Taken together, these various locations of AII-positive cells and terminals would explain the neuromodulator/neurotransmitter roles (circumventricular organ), the neuroendocrine role (supraoptic, paraventricular nuclei; neurohypophysis), and the paracrine/autocrine role (gonadotroph of the adenohypophysis) subserved by AII.

Neuropeptide Y (NPY) is a highly conserved 36 aa peptide that is widely distributed in the central nervous system in many mammals. Of particular importance to hypothalamic function are the extensive networks of NPY-positive fibers and terminals within the medial preoptic area, the periventricular and anterior hypothalamic areas, the suprachiasmatic, supraoptic, and paraventricular nuclei, the arcuate and ventromedial nuclei, as well as the median

eminence. Significant concentrations of NPY are found in hypophyseal portal blood. Within the hypothalamus, NPY-positive cells are distributed in the arcuate nucleus/median eminence area and in the dorsomedial nucleus. Interestingly, much of the hypothalamic NPY originates from noradrenergic cells outside of the hypothalamus. These cells are found in the lateral reticular medulla, the nucleus tractus solitarius region, the locus coeruleus and subcoeruleus. Transection of ascending noradrenergic fibers does not eliminate but substantially decreases NPY immunoreactivity in various hypothalamic areas. NPY has multiple actions within and outside the central nervous system. Most striking is its effect on the adenohypophysis and on some behaviors. Specifically, NPY is a major orexigenic peptide, stimulating appetite and food intake (described in Section 4.3.1.3). NPY also plays a role in the regulation of gonadotropin secretion by acting within the hypothalamus as a neuromodulator/neurotransmitter affecting GnRH secretion as well as a neurohormone affecting LH secretion directly. NPY has also been implicated in the control of secretion of ACTH, growth hormone, and prolactin from the pituitary gland. It may act as a neurotransmitter affecting the secretion of AVP from the neurohypophysis. Moreover, NPY is synthesized in a subpopulation of thyrotrophs in the adenohypophysis suggesting a paracrine/autocrine role. Finally, NPY controls eating behaviors through intrahypothalamic pathways.

Orexin (also known as hypocretin) is a 33 amino acid peptide identified in the lateral hypothalamus, an area long associated with the stimulation of food intake. There are two forms of the peptide, orexin A (hypocretin 1) and orexin B (hypocretin 2), of which orexin A seems to be the most important regulator of hypothalamic function. Orexin appears to be important for overall levels of arousal, with lack of orexin associated with narcolepsy (excessive daytime sleepiness). It also seems to play a specific role in the stimulation of food intake.

Galanin is a highly conserved 29 amino acid peptide that is widely distributed throughout the central and peripheral nervous system. Within the hypothalamus, galanin-positive cell bodies are found in the supraoptic and paraventricular nuclei as well as the arcuate nucleus. Dense efferent fibers from these areas terminate in the external and internal layer of the median eminence as well as in the neurohypophysis. Galanin-like immunoactivity and its message is expressed in somatotrophs, lactotrophs, and thyrotrophs within the adenohypophysis. The

expression in thyrotrophs and expression in lactotrophs is positively regulated by thyroid hormones and estrogen, respectively. Galanin has been implicated as a neurotransmitter/neuromodulator in the control of growth hormone, ACTH, TSH, LH, and prolactin secretion. In fact, it has been found to colocalize with CRH and GnRH in the hypothalamus. Galanin is secreted from cells of the adenohypophysis and is present in portal blood. Taken together, galanin affects pituitary hormone secretion as a neurotransmitter/neuromodulator, as a neurohormone, and as a paracrine/autocrine factor.

Endothelins (ETs) are a family of regulatory peptides with vasoconstrictor activity originally isolated from incubation media of vascular endothelial cells. One, designated ET-1, is a 21-residue peptide that contain 2 intramolecular disulfide bonds. Two related peptides, designated ET-2 and ET-3, differ by 2 and 6 amino acid residues, respectively. The localization of the ETs in the supraoptic and paraventricular nuclei of the hypothalamus, as well as the adenohypophysis and neurohypophysis, and the presence of ET-receptors in the hypothalamus as well as the adenohypophysis and neurohypophysis has prompted active inquiry into the role of the ETs in pituitary hormone secretion. In general, the ETs act within the hypothalamus to enhance LH secretion via stimulation of GnRH. Within the pituitary, they are capable of stimulating LH, FSH, TSH, and ACTH secretion, directly. In addition, they are potent inhibitors of prolactin secretion but exert no action on growth hormone secretion from the pituitary gland. It is not known if the ETs are in the portal circulation. These data suggest that the ETs can act as neuromodulators/neurotransmitters or even in a paracrine/autocrine manner to affect pituitary hormone secretion.

2.5.5. MONOAMINES

In addition to the peptide neuromodulators, described above, the hypothalamus receives significant innervation from classic monoamine neurotransmitters. There are essentially three monoamines of importance to hypothalamic function whose distributions have been described:

Dopamine: The direct neuroendocrine role of the tuberoinfundibular and tuberohypophyseal dopamine neurons have been discussed above. In addition, dopamine plays an important neuromodulatory role in the hypothalamus. There are two major dopaminergic systems with long axons that originate from outside the hypothalamus (Fig. 21). One of these, the *nigrostriatal system*, has cell bodies in the substantia

nigra with long axons that terminate in the caudate-putamen and globus pallidus. A second, the *mesolimbic system*, has cell bodies in the ventral tegmentum that send projections through the hypothalamus and terminals in areas of the limbic system such as the nucleus accumbens, olfactory tubercle, cingulate cortex, and frontal cortex. Axons of both of these areas travel through the medial forebrain bundle. The hypothalamus also contains an intrinsic dopaminergic pathway with short axons. The cell bodies of the *incertohypothalamic neurons* are located in the caudal hypothalamus, zona incerta, and rostral periventricular nucleus with axons terminating in the dorsal hypothalamus, preoptic area, and septum. Within the hypothalamus, it seems that the incertohypothalamic dopaminergic neurons play a neuromodulatory role whereas the tuberoinfundibular and tuberohypophyseal neurons subserve a neuroendocrine role, described above.

Norepinephrine: The noradrenergic cell bodies of greatest importance to the hypothalamus is the *locus coeruleus* (Fig. 22). The axons course toward the hypothalamus as the large *dorsal noradrenergic (or tegmental) bundle* and the *rostral limb of the dorsal periventricular pathway*. The former pathway joins the ascending *ventral noradrenergic bundle* from the *lateral tegmental noradrenergic cell groups*. The dorsal and ventral noradrenergic pathways unite in the *median forebrain bundle* to enter the amygdala (dorsal) and hypothalamus (ventral).

Serotonin: Two groups of serotonergic cell bodies are found in the brain in the *dorsal and medial raphe nuclei* (Fig. 23). Axons from the dorsal raphe nucleus form the *ventral ascending serotonergic pathway* that sweeps ventrally then curves rostrally through the ventral tegmentum to join noradrenergic fibers of the median forebrain bundle in the lateral hypothalamic area. Two large fiber groups leave the ventral ascending pathway as it courses through the lateral hypothalamus: one directed laterally, the other ventromedially. The ventromedial fibers innervate a large number of hypothalamic areas including the lateral, medial preoptic, and anterior hypothalamic areas as well as the dorsomedial and ventromedial nuclei, the infundibulum, and the suprachiasmatic nuclei. In addition, the OVLT is rich in serotonin terminals. Recent work has shown that serotonin neurons provide primary afferents to the GnRH neurons. The serotonin neurons in the dorsal raphe contain receptors for estrogen and thus may represent a significant indirect pathway for estrogen actions on GnRH neurons.

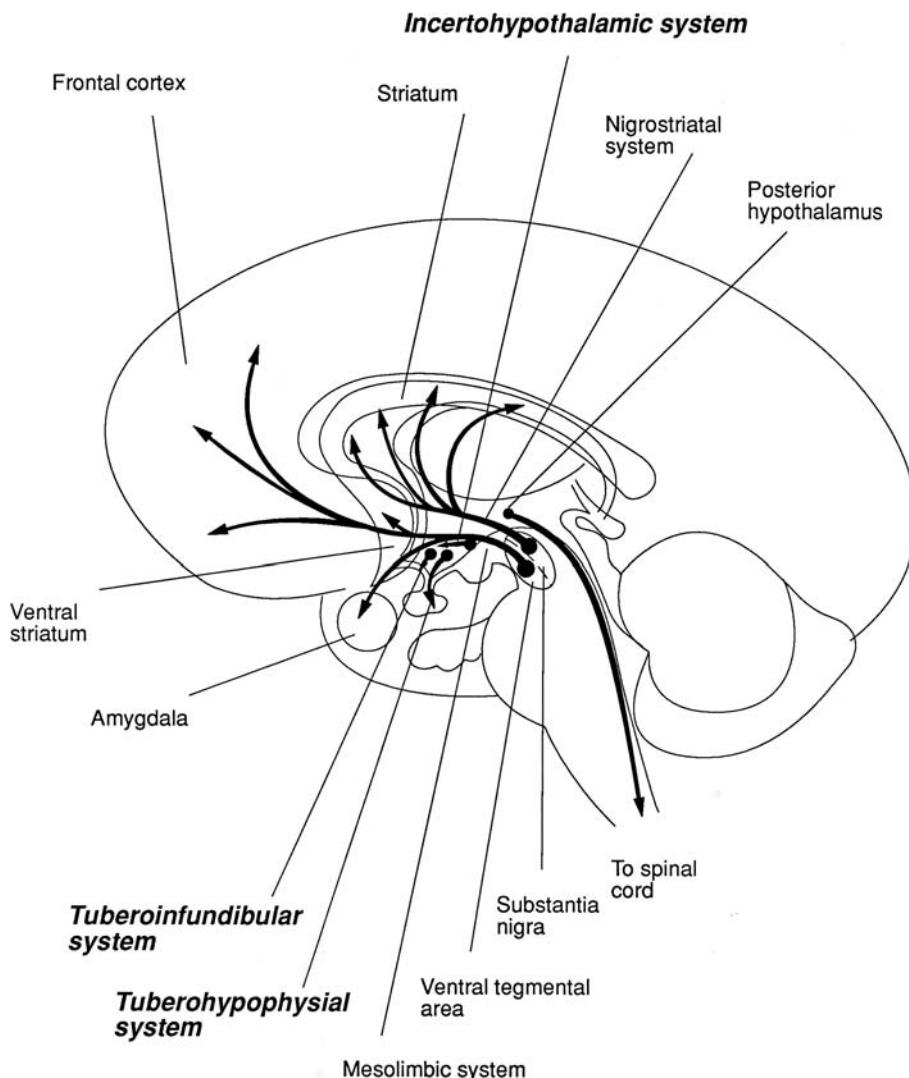


Fig. 21. Diagrammatic representation of the dopaminergic system. Two dopaminergic systems originate and terminate outside of the hypothalamus: the *nigrostriatal* and *mesolimbic*. The hypothalamus contains three intrinsic dopaminergic systems: (1) the *incertohypothalamic* with cell bodies in the caudal hypothalamus, zona incerta, and rostral periventricular nucleus with terminals in the rostral preoptic area and septum; (2) the *tuberoinfundibular* with cell bodies in the arcuate and periventricular nuclei and terminals in the external zone of the median eminence adjacent to the primary capillary bed; (3) the *tuberohypophyseal* with cell bodies in the rostral arcuate and periventricular nuclei and terminals in the intermediate and posterior lobe of the pituitary gland. The tuberoinfundibular and tuberohypophyseal dopaminergic systems are those responsible for delivering dopamine to the adenohypophysis through the portal vasculature.

3. TECHNIQUES FOR STUDYING HYPOTHALAMIC FUNCTION

Many techniques have been employed to study hypothalamic function, through investigation of the location and activity of hypothalamic neurons. Below is a brief description of some of the approaches available to give some insight into the approaches used.

3.1. Anatomic Techniques

Anatomic techniques allow the identification of specific populations of neurons, either by characterizing the neurochemical phenotype of those neurons or by determining whether specific neurons show changes in activity in response to a stimulus (Table 11). They have the shortcoming that they are usually only single snapshots in time, as the tissue processing

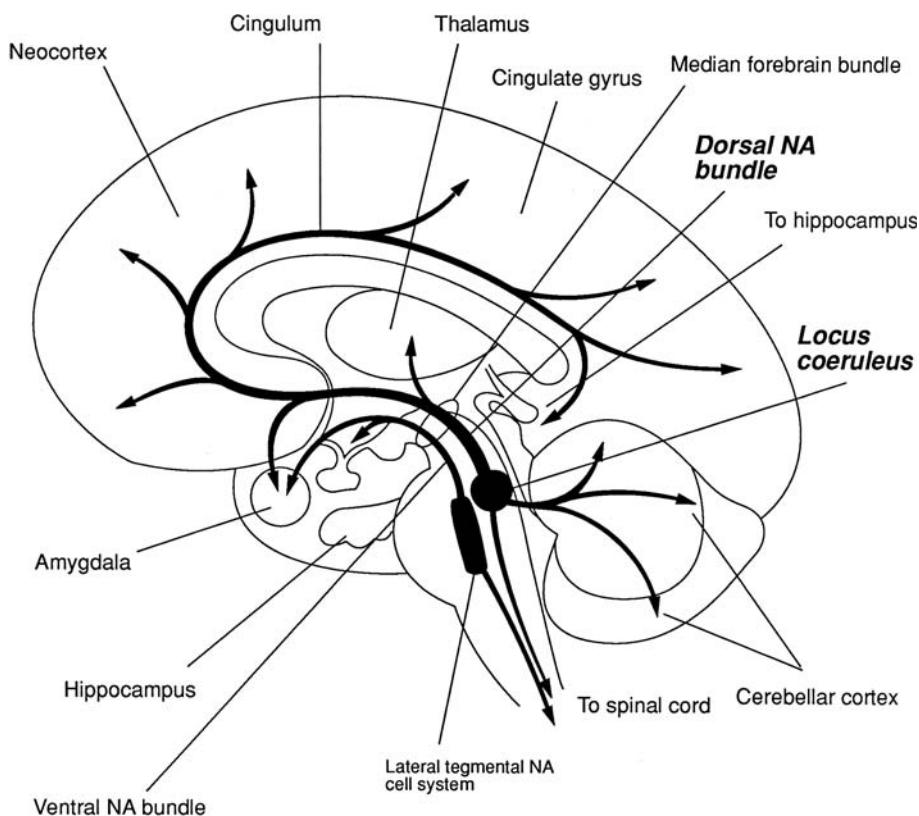


Fig. 22. Diagrammatic representation of the noradrenergic system. The cell bodies of greatest importance to the hypothalamus are located in the locus coeruleus whose efferents course toward the hypothalamus as the dorsal noradrenergic bundle. They terminate in the paraventricular and arcuate nuclei of the hypothalamus as well as in the preoptic area.

required precludes use in intact animals. However, by combining approaches, it is possible, for example, to detect whether a particular identified neuronal population expresses receptors for a hormone and is activated in response to administration of that hormone.

3.1.1. IMMUNOHISTOCHEMISTRY

Immunohistochemistry is a common approach for identifying hypothalamic neurons, visualizing neuropeptides or their receptors in neuronal cell bodies, dendrites, axons, and axon terminals. The technique involves labeling histologically prepared sections of hypothalamus with antibodies that are specific for the target neuropeptide or receptor. The antibodies are then visualized, usually using secondary antibody systems conjugated to an enzyme that will develop a visible precipitate, or to a fluorophore that will produce a distinctive fluorescent color. Localization of peptide in different parts of neurons requires different approaches and produces different information. For example, to study neuropeptides in cell bodies, it is often necessary to block axonal transport with agents

such as colchicine that disrupt microtubules. Cell body density can then be estimated. Topographical three-dimensional localization of neuropeptide-containing neurons can be determined. Axons and nerve terminals may require electron microscopy to view adequately. Immunohistochemistry can still be used but will require using an electron-dense marker, such as gold particles, conjugated to the secondary antibody.

3.1.2. TRACT TRACING

Tract tracing techniques can be employed to determine the path taken by axons of particular neurons. A dye or label is microinjected into the region of interest, and then the presence of that dye in other regions of the brain provides evidence of neuronal connectivity between the two regions. Tracts can be visualized from the neuronal cell body to the axon terminal (anterograde) or from the nerve terminal to the cell body (retrograde). For example, horseradish peroxidase is a glycoprotein enzyme that is taken up by neurons and can be used for both anterograde and retrograde tracings. Fluorogold is a fluorophore that

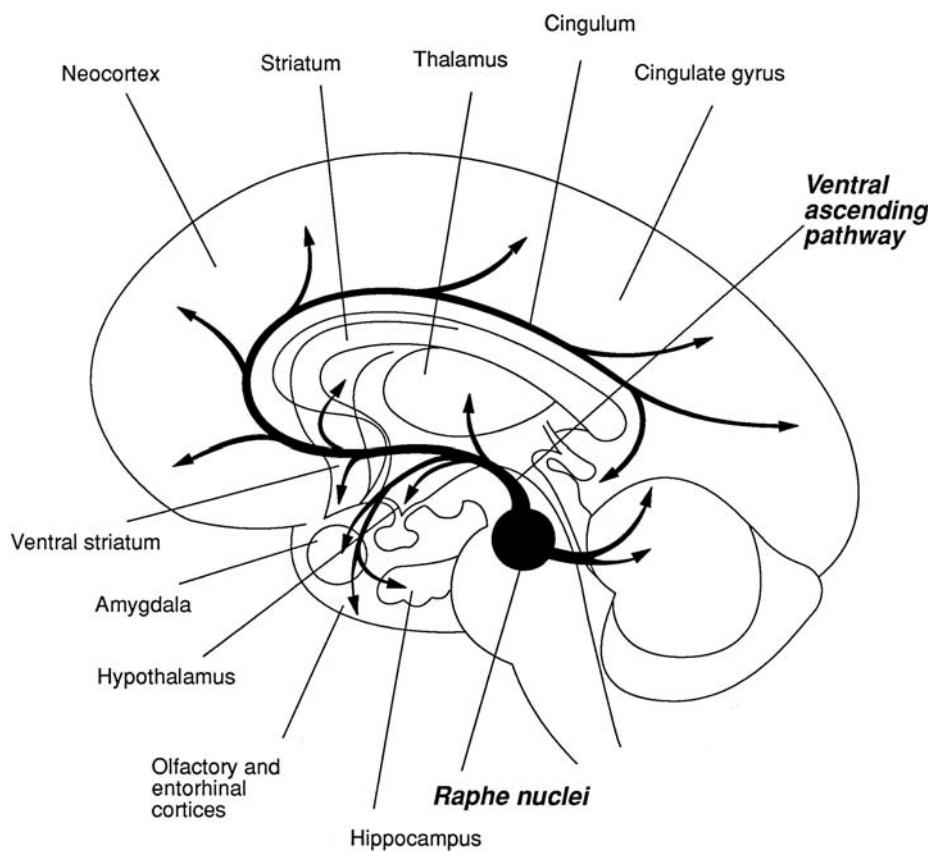


Fig. 23. Diagrammatic representation of the serotonergic system. The cell bodies of importance to hypothalamic function are found in the dorsal and medial raphe nuclei. Axons from the dorsal raphe form the ventral ascending serotonergic pathway and enter the hypothalamus ventromedially to terminate in the anterior hypothalamus, dorsomedial and ventromedial nuclei, the suprachiasmatic nuclei, and the infundibulum. In addition, serotonergic terminals are found in the lateral and medial preoptic areas as well as the OVLT.

is used specifically for retrograde tracings, and Phaseolus vulgaris leukoagglutinin or biotinylated dextran amines are specifically transported anterogradely along axonal tracts. Combined with immunocytochemistry to identify neuropeptides or their

receptors, tract tracing methods may be used to characterize the microcircuitry of specific neural systems.

3.1.3. AUTORADIOGRAPHY

Autoradiography is a technique that uses an image produced on x-ray film or photographic emulsion to identify radioactively labeled compounds in a histologic section. Typically, it is used to identify hormone or neuropeptide receptors, using a radioactively labeled hormone or peptide to bind to the receptors, and then apposing the labeled tissue to the film or emulsion. The radioactivity is detected through photographic development of the film or emulsion. The process is termed *in vivo autoradiography* if the ligand is administered into the circulation (with subsequent tissue removal and sectioning) or *in vitro autoradiography* if the ligand is incubated with the tissue sections. The ligands are generally labeled with ^3H (tritium) or ^{125}I .

Table 11
Anatomic Techniques for Studying Hypothalamic Function

Methods	Use
Immunohistochemistry	Visualize location of proteins, including peptides and their receptors, identifying active neurons
Tract tracing	Visualize axons, map neural networks
Autoradiography	Identify receptors and binding sites of peptides

3.1.4. MARKERS OF NEURONAL ACTIVITY

Neurons can also be labeled for activity. For example, the protein product, Fos, of the immediate early gene c-fos can be detected by immunohistochemistry in the nuclei of acutely activated neurons. In a typical experiment, an acute manipulation will be completed (e.g., administration of a peptide) and the brains collected 1 to 2 h later. Neurons that have responded to that manipulation would show an acute activation of Fos expression, identified as Fos immunoreactivity in the nucleus of the neuron. Numbers of responsive neurons in specific brain regions can be quantified as an index of the neuronal response to the specific manipulation. Similarly, activation of specific transcription factors (e.g., phosphorylation of specific signal transduction molecules such as pCREB) can be used to identify neurons that have responded to a particular hormone or neurotransmitter. In that case, antibodies that are specific to the phosphorylation status of a particular molecule would be used.

3.2. Physiologic Techniques

These are approaches that can generally be applied in living animals or living tissues (Table 12). This has the key advantage that it is possible to observe changes in function over time (e.g., before and after a particular treatment).

3.2.1. RADIOIMMUNOASSAY

One of the classic techniques in neuroendocrinology is the *radioimmunoassay (RIA)*, which can be used to accurately quantify concentrations of a compound either in a tissue sample or a blood sample or cerebrospinal fluid. This technique was so influential that Rosalind Yalow shared the Nobel Prize for Physiology or Medicine in 1977 for the development of the RIA. RIAs involve competition between unlabeled hormone or peptide in a sample and a known amount of radioactively labeled hormone for binding

to a restricted amount of a highly specific antibody to that compound. The higher the concentration of the target ligand, the less of the radioactive competitor will be bound. The unknown samples can then be compared with a standard curve prepared from known concentrations of ligand, providing an extremely sensitive and accurate measure of the amount of ligand in a sample. This technique has been routinely applied to monitoring pituitary hormones in blood, providing an indirect estimate of the activity of neurosecretory neurons in the hypothalamus. For example, measuring levels of LH in frequent blood samples (5 to 10 min) will reveal expression of LH pulses, which reflect the pulsatile release of GnRH from the hypothalamus.

3.2.2. SAMPLING OF PITUITARY PORTAL BLOOD

For a more direct measure of activity of hypothalamic neurosecretory neurons, it is also possible to measure the concentration of biogenic amines and neuropeptides in the microscopic vessels, the hypothalamo-hypophyseal portal vessels connecting the median eminence with the adenohypophysis. Collection of the blood in these vessels free from dilution by peripheral blood involves a complex ventral surgical approach to expose the median eminence. Once exposed, the stalk is cut and placed inside a polyethylene cannula. Using this procedure, blood is collected from all of the cut portal vessels simultaneously, and hormone levels can be measured by RIA. In laboratory rodents, this procedure involves experimental manipulation and collection of portal blood under anesthesia and hence is limited in its interpretation. In larger mammals, however, surgical approaches have been developed that allow portal blood to be collected from unanesthetized sheep, horses, pigs, and monkeys. In these animals, it is possible to monitor hypothalamic hormone secretion in conscious animals throughout physiologic processes, such as during exposure to stress or during ovulation.

Table 12
Physiologic Techniques for Studying Hypothalamic Function

Methods	Use
Radioimmunoassay	Quantify levels of hormones in blood
Portal blood sampling	Measure hormone release from hypothalamic hypophysiotropic neurosecretory neurons
Lesions	Identify functions of hypothalamic areas by inducing deficits
Electrophysiology	Measure activity of live hypothalamic neurons, either <i>in vivo</i> or <i>in vitro</i> slice preparation

3.2.3. HYPOTHALAMIC LESIONS

One approach that has been extremely productive in defining hypothalamic function is to surgically remove or damage small parts of the hypothalamus to create deficit syndromes, and then study the effect on hormone secretion or behavior. The first example of this approach was the technique of *hypophysectomy*, or surgical removal of the pituitary gland from the sella turcica by a parapharyngeal approach, described in the rat by Philip Smith in 1927. Because the hypothalamus controls pituitary function, hypophysectomy creates many, but not all, of the deficits of hypothalamic hypofunction. In early research, the removed gland was *transplanted* to sites distant from the hypothalamus. Despite revascularization of the transplanted pituitary, deficit symptoms of all the hormones except prolactin persisted, demonstrating that hypothalamic stimulation is required to induce pituitary hormone secretion. The hyperprolactinemia seen in this situation was evidence that the predominant regulation of prolactin secretion by the hypothalamus is one of inhibition. When the pituitary gland was transplanted back to the sella turcica to allow revascularization by the hypophyseal portal vessels, the deficit symptoms were reversed. These types of procedures allowed early anatomists to conclude that the critical link between the hypothalamus and adenohypophysis was *neurovascular*.

To more specifically investigate functions of individual hypothalamic nuclei, small *lesions* can be experimentally induced within the hypothalamus. To accurately locate the nuclei, a surgical approach known as stereotaxic surgery is required. The head of the subject is placed in a stereotaxic frame, which holds it in a predefined, rigid position. With the aid of a map of the brain (a stereotaxic atlas), as well as anatomic landmarks on the skull and the surface of the underlying brain, electrodes can be placed accurately within a hypothalamic nucleus. Focal lesions that destroy discrete anatomic groupings of cell bodies can be placed by sending a current through this electrode. Similarly, fibers of passage may be destroyed by stereotaxic placement of a small knife, which can be manipulated to cut specific fiber tracts controlling the hypothalamus. Selective lesions can also be induced by neurotoxins administered stereotactically into specific brain regions. For example, *monosodium glutamate* or its more potent analogue, *kainic acid*, selectively damage neuronal cell bodies and dendrites, sparing fibers of passage and axon terminals. This allows identification of the specific function of a hypothalamic nucleus by studying the

loss of that function after lesion. These compounds can also be administered systemically to selectively damage areas outside of the blood/brain barrier such as the arcuate nucleus and median eminence, the OVLT, and the preoptic area. A classic example of the use of this technology is the administration of *gold thioglucose*. This drug poisons the glucose-sensitive cells in the ventromedial nucleus of the hypothalamus. The result is the development of profound obesity, clearly demonstrating a functional role of this nucleus in the suppression of food intake.

Neurotoxic lesions can be made in neurotransmitter-specific manner, using drugs that are selective for a particular neurotransmitter system. For example, in catecholamine neurons, *6-hydroxydopamine* or *6-hydroxydopa* will deplete neurotransmitter stores. These compounds pass the blood-brain barrier so their specificity may be restricted by stereotaxic injection in small volumes locally. Similarly, the indolamine neurotoxins, *5,6-* or *5,7-dihydroxytryptamines*, will specifically impair serotonin production, and *cysteamine* and *2-mercaptoethylamine* deplete somatostatin in both cell bodies and axons. Certain plant lectins, such as *ricin*, are taken up and transported retrogradely along axons to cell bodies and ultimately kill the cell. This characteristic has been used to specifically target populations of hypothalamic neurons. Specificity is conferred by coupling the toxin to an antiserum to the peptide made by the cell so that when the antiserum binds to the peptide, specific cells are killed. Alternatively, the cytotoxic plant lectins can be conjugated to hypophysiotropic peptides to selectively kill target cells.

3.2.4. ELECTROPHYSIOLOGY

Electrophysiologic studies of the hypothalamus have provided a great deal of information on the firing patterns of hypothalamic neurons. However, this information is most valuable when the firing patterns are correlated with a hypothalamic-dependent event such as pituitary hormone secretion or behavioral responses. In general, hormone release from cells or neuropeptide release from axon terminals in the hypothalamus follows influx of calcium through membrane depolarization during action potential activity.

Extracellular recordings from hypothalamic nuclei *in vivo* can be used for topographic or functional identification. For example, magnocellular neurons in the paraventricular nucleus can be excited antidromically and identified by electrically stimulating the neurohypophysis. The topographic origin of those neurons terminating in the

neurohypophysis can be identified by this means. Similarly, these neurons can be recorded orthodromically after application of a suckling stimulus and correlated with the release of oxytocin into peripheral plasma. Such a relationship suggests, but does not prove, a functional role for the neurons recorded in control of pituitary hormone secretion.

Several *in vitro* electrophysiologic approaches can be used to study neurotransmitter effects on hypothalamic neurons or effects of hypophysiotropic substances of hypothalamic origin on target cells. Slice preparations of whole hypothalami allow for introduction of drugs or other factors by superfusion close to the neuron being recorded. Excitable membrane properties of pituitary cells can be studied after application of suspected neurohormones of hypothalamic origin. A complication with such studies is the specific identification of the neurons from which recordings are being made. Many hypothalamic nuclei contain mixed populations of cells, and it is difficult to be certain, just from firing characteristics of the cell, what type of neuron is being recorded. In recent years, this has been addressed by use of transgenic animals (see later). *Voltage clamp* or *current clamp* approaches are also used to study the effects of hypophysiotropic substances on secretory function of pituitary cells. These yield valuable information about the identity of ion channels involved in secretion.

3.3. Neurochemical Techniques

In many cases, studies of hypothalamic function involve examining classic neurotransmitters and neurotransmitter release from nerve terminals, as well as measuring hypothalamic peptides and hormones. Analysis of these pathways often requires slightly different techniques and has led to the development

of some innovative new ways of analyzing hypothalamic function (Table 13).

3.3.1. TISSUE CONTENT OF NEUROPEPTIDES OR NEUROTRANSMITTERS

The content of neurotransmitters and neurohormones in hypothalamic tissue can be informative. Though there are several *brain microdissection techniques* available, a particularly useful approach is the *micropunch technique*. Specific brain regions, as small as a few hundred micrometers in diameter, can be punched from fresh or frozen sections of brain using needles constructed from stainless steel tubing. The punched tissue is then placed into a tube for subsequent homogenization and extraction of the compounds of interest. For measurement of neuropeptides, an RIA might be used. For measurement of neurotransmitters, such as the catecholamines or GABA, content is commonly measured using *high-performance liquid chromatography* coupled with *electrochemical detection (HPLC-EC)*. Using this technique, separation of neurotransmitters is achieved with an analytical column packed with C₁₈ reverse-phase material. Using slightly different conditions, this procedure can be adapted to measuring catecholamines, monoamines and their metabolites, or amino acids such as GABA and glutamate. Resolution of sample molecules takes place by their differential interactions with the mobile phase solvent and the column packing material. Distinct bands of solute form during passage through the column. Resolution of the solutes are controlled by pH, ionic strength, nature and concentration of aqueous phase, and the concentration of the organic components of the mobile organic phase. Quantitation is achieved by eluting the resolved solutes through the electrochemical detector. The potential applied to the detector's cell favors oxidation of the neurotransmitter. For a given set of operating conditions, the oxidative

Table 13
Neurochemical Techniques for Studying Hypothalamic Function

Methods	Use
Microdissection	Isolate specific hypothalamic nuclei for quantitation of neuropeptides and neurotransmitter concentration
<i>In vivo</i> voltammetry	Monitor changes in levels of catecholamine neurotransmitters in live tissue
Microdialysis and push-pull perfusion	Measure peptide or amine dynamics in brain tissue or CSF

current is directly proportional to the concentration of electroactive species in solution.

Activity of catecholamine neurons is often estimated by calculating the ratio of a catecholamine to its metabolites. Alternatively, neurotransmitter turnover can be estimated by using pharmacologic treatments to block synthesis, and measuring the rate of depletion of the neurotransmitter from the tissue, or to block degradation, and measure the rate of accumulation in the tissue. These “active” measurements are more informative than the simple “steady state” concentration of a neurotransmitter, because even large changes in neuronal activity might not affect the overall concentration of a neurotransmitter in the tissue. For example, if synthesis, release and degradation of the neurotransmitter were all increased, there would be a dramatic increase in neurotransmitter turnover but relatively little effect on overall concentration of neurotransmitter in the tissue.

3.3.2. *In Vivo* VOLTAMMETRY

Related to the HPLC-EC procedure is a method using electrochemistry for determining catecholamine flux *in vivo* in neural tissue. This procedure, known as *in vivo voltammetry*, involves stereotaxic placement of a carbon-based microelectrode into a specific hypothalamic region. As a potential is applied to the electrode and increased, the catecholamines in a thin surface adjacent to the electrode are oxidized. The magnitude of the oxidizing current generated is a function of the concentration of electroactive species in solution. The potential at which the current appears is specific for particular catecholamines. Unfortunately, this technique cannot differentiate subtle differences in side-chain groups and thus dopamine, norepinephrine, and epinephrine cannot be adequately differentiated.

3.3.3. MICRODIALYSIS AND PUSH-PULL PERfusion

Although the micropunch technique represents a highly useful method for estimating tissue content of neurotransmitters and neurohormones, its utility is limited by the fact that a single animal can only be sampled at a single point in time. This has been overcome by two related methodologies: *push-pull perfusion* and *microdialysis*. Each method has the distinct advantage of multiple sampling over time. To use push-pull perfusion, concentric stainless steel cannulae are implanted so that the tip of the inner cannula is located at the desired site of study (Fig. 24). Artificial cerebral spinal

fluid (CSF) is “pushed” through the inner cannula and instantaneously “pulled” through the outer cannula into an appropriate receptacle. Assuming the push-pull rates are matched, the “pulled” CSF should sample the extracellular space at the tip of the probe, allowing neuropeptides and transmitters in this region to be quantified. A refinement of the push-pull perfusion method, microdialysis, has been likened to the implantation of an artificial blood vessel in tissue. A probe bearing a small piece of semipermeable dialysis membrane at the end is implanted into the hypothalamus. The end is localized in the area of interest for study. Artificial CSF or saline is pumped through the probe and recovered. Theoretically, amines or peptides will diffuse from the area of higher concentration in the brain to the area of lowest concentration on the probe side of the dialysis tubing. The size-exclusion selectivity of the membrane will determine the size of the molecule diffusing into the probe, and the length of the membrane will determine the amount of tissue sampled. Concentrations of peptides and neurotransmitters can then be quantified, using the methods described above. The major limitations on this method are the size of the probes, relative to the areas being sampled, and the relatively low recovery of substances into the dialysis fluid.

3.4. Molecular Techniques

With the advent of modern molecular biological techniques, it is possible not only to measure the amount of peptide in the hypothalamus but also to measure the regulation of neuroendocrine peptide gene expression by quantitation of specific messenger RNA and to regulate levels of gene expression to examine the effects on hypothalamic function (Table 14).

3.4.1. MICRODISSECTION AND QUANTITATION OF mRNA LEVELS

The most commonly used approach to quantify mRNA is to isolate the hypothalamus or specific microdissected regions of the hypothalamus, as described above, and then extract RNA from this sample. There are a variety of approaches that can be taken to quantifying specific mRNA molecules. Classically, mRNA was quantified using hybridization assays such as the Northern blot, in which RNA molecules are separated by electrophoresis and then labeled with a radiolabeled complementary RNA probe, or ribonuclease protection assays, in which a

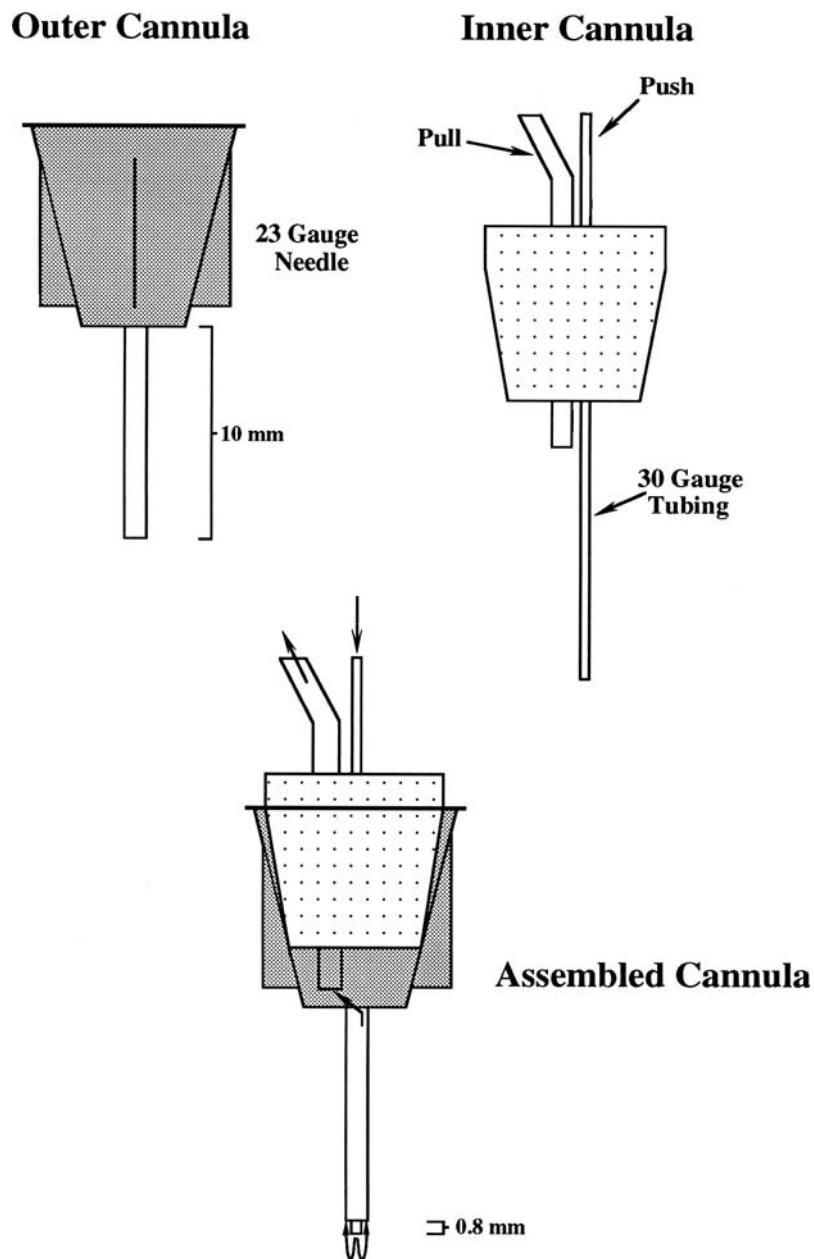


Fig. 24. The structure of the inner, outer, and assembled push-pull perfusion cannula. The *arrows* indicate the direction of flow through the assembled cannula.

Table 14
Molecular Techniques for Studying Hypothalamic Function

Methods	Use
Northern blotting, nuclease protection assays, real-time RT-PCR	Quantifying levels of mRNA in tissue
<i>In situ</i> hybridization	Visualize mRNA expression in histologic sections
Antisense oligonucleotides and RNAi	Inhibit gene expression

radioactively labeled complementary strand of RNA is hybridized with the target mRNA, protecting it from subsequent degradation by ribonuclease enzymes. The protected fragments are then separated by polyacrylamide gel electrophoresis and quantified by autoradiography. A more common approach now is to use real-time reverse transcriptase–polymerase chain reaction (RT-PCR). This is a more strictly quantitative variant of the RT-PCR procedure, which involves reverse transcribing RNA in the sample to complementary DNA (cDNA), and then using sequence specific primers to select the cDNA of interest, exponentially amplifying the cDNA by PCR. In real-time RT-PCR (also known as quantitative PCR or qPCR), the amplified products are continuously measured throughout the amplification process. This means that no matter what starting concentration of cDNA in the sample, the reaction can always be measured in the linear phase of amplification. This is a highly sensitive method for quantifying mRNA in a sample, sensitive enough to detect mRNA even from a single cell. The shortcoming of these methods is that they require a start point of homogenized tissue for RNA extraction and thus lose any anatomic resolution. One novel method that begins to improve on this is the use of the laser capture microdissection microscope. With this tool, it is possible to cut specific nuclei from tissue sections, either labeled with histologic stains or by immunohistochemistry, using a laser. The sensitivity of the qPCR approach allows detection of mRNA even in very small pieces of tissue collected in this manner.

3.4.2. *IN SITU HYBRIDIZATION*

The best approach to measuring mRNA levels while retaining anatomic resolution, however, is a method known as *in situ hybridization histochemistry*. This is the “mRNA equivalent” to measuring localization of a protein by immunohistochemistry. In this case, specific complimentary RNA or DNA probes are used in place of an antibody. Radiolabeled complementary RNA or DNA probes are prepared and then incubated with the tissue sections. The probes will hybridize with mRNA in the section. Unhybridized probe is then washed off, and the labeled mRNA can be visualized using autoradiography against x-ray film or, more commonly, in photographic emulsion placed directly on the section. Typically, positive signal will appear under the microscope as silver grains located in the developed photographic emulsion. Alternatively, nonradioactive probes are now available, allowing visualization of a

chromogenic reaction using an immunohistochemical approach to detect the RNA or DNA probe.

3.4.3. INHIBITION OF GENE EXPRESSION

Previous studies of actions of some hypothalamic peptides have been limited by the lack of availability of specific pharmacologic compounds to inhibit their action *in vivo*. Modern molecular techniques have now provided an alternative to the use of drugs to regulate peptide function. There are now several ways to regulate gene expression *in vivo*, allowing the specific inhibition of expression of neuropeptide or its receptors for subsequent investigation of loss of function. *Antisense oligonucleotides* are single strands of DNA that are complementary to a chosen sequence and have been used to block expression of specific genes. They are stereotactically injected into a hypothalamic region of interest and are thought to act either by providing a translation block, preventing translation from the targeted mRNA, or by forming a DNA/RNA hybrid with the targeted mRNA, causing it to be degraded by the enzyme RNase H. Working on a similar principle, *RNA interference* (abbreviated RNAi) is a mechanism for RNA-guided regulation of gene expression, in which double-stranded RNA molecules inhibit the expression of genes with complementary nucleotide sequences. Specific RNAi molecules can be designed to inhibit expression of target genes, then the molecules delivered into the brain by stereotaxic injection, as described above. An endogenous dicer enzyme cleaves the double-stranded RNAi molecules to short double-stranded fragments of 20 to 25 base pairs, which are then incorporated into the complementary RNA sequences in the mRNA within the cell, inducing its degradation. Thus, this represents a form of posttranscriptional gene silencing. The selective action of antisense oligonucleotides and RNAi on gene expression makes them valuable research tools. Whereas some studies have injected antisense oligonucleotides or RNAi molecules directly into the brain, a more efficient way of delivering these molecules is by using adenoviruses, or adeno-associated viruses (AAV). These are small, replication-deficient mammalian viruses that are being used for gene therapy. They can infect and deliver genes into adult mammalian cells, including neurons. Adenoviruses have also been used to overexpress compounds of interest in the hypothalamus, an equally useful tool for investigating hypothalamic function.

3.5. Behavioral Techniques

Until now, we have focused on the neuroendocrine functions of the hypothalamus. It is important to recognize the significant role that the hypothalamus plays in important behaviors, including reproductive behavior, aggression, food seeking, pair bonding, and maternal behavior. After manipulations to the hypothalamus, for example by lesions or specific modulation of gene expression, as described above, behavioral phenotyping plays an important role in characterizing the effects of those manipulations. Behavioral testing is completed under extremely controlled conditions, with independent tests designed to specifically test certain aspects of behavior.

3.6. Transgenic Techniques

The advent of capability for genetic manipulation of mice has revolutionized certain aspects of biomedical research. The application of these techniques to hypothalamic research is expanding at a rapid rate, with more and more novel and innovative approaches being produced all the time. The technology provides the ability to manipulate gene expression *in vivo*, either overexpressing a gene or deleting a specific gene from the genome. We also have the capability to label specific cells in the hypothalamus, making it easier to conduct other types of research. Though at present the vast majority of work has been restricted to transgenic mice, there are a few reports of transgenic modifications in other species including rats and sheep (Table 15).

3.6.1. PROMOTER TRANSGENICS

These are relatively straightforward transgenic manipulations, in which a specific gene sequence attached to the promoter sequence from another gene is injected into the pronucleus of fertilized oocytes, and then these oocytes are returned into the uterus of pseudopregnant female mice and allowed to develop. For example, a common application is to introduce a reporter gene, such as green fluorescent protein (GFP), under the control of a hypothalamic neuron specific

promoter, such as GnRH, POMC, or NPY. This results in a line of mice in which GFP is expressed specifically within the target population of hypothalamic neurons. This offers a range of new experimental opportunities. For example, the GFP-labeled neurons can be identified in a living brain slice, allowing electrophysiologic investigation of an identified population of cells. It also allows identification of neurons that might express very low levels of a protein that would be difficult to detect using other methods.

3.6.2. GENE KNOCKOUTS

A knockout mouse is a genetically engineered mouse that has had one or more genes deleted. This is most commonly achieved by a process known as homologous recombination in embryonic stem cells. An artificial piece of DNA is generated that contains a modified sequence with critical pieces of a gene sequence missing, but which shares identical, or homologous, sequence to the gene of interest. This modified sequence is introduced into the stem cells using a viral vector. The homologous sequence flanks the existing gene's DNA sequence both upstream and downstream of the target gene's location on the chromosome. The cell's own nuclear machinery recognizes the identical stretches of sequence and replaces the existing gene with the artificial piece of inactive DNA, knocking out the function of the original gene. After the artificial DNA is inserted, the genetically altered embryonic stem cells are grown *in vitro* for several days before being injected into mouse blastocysts. The blastocysts are implanted into the uterus of a pseudopregnant female mouse and allowed to develop. The resulting pups are chimeric, in that they have some tissues in which a gene has been knocked out—those derived from the altered ES cells. However, they also have some normal tissues derived from the nonaltered embryos into which the altered ES cells were injected. It is necessary to extensively cross-breed such mice to generate homozygous knockouts. Knockout mice provide a powerful tool

Table 15
Transgenic Techniques for Studying Hypothalamic Function

Methods	Use
Promoter transgenics	Label hypothalamic neurons with reporter molecules, e.g., GFP; overexpress a protein of interest in the brain
Gene knockouts	Investigate function of a gene by establishing a deficit
Conditional gene knockout	Investigate function of a gene by establishing a deficit in a time- or tissue-specific manner

to evaluate the function of a specific gene. They have the significant limitation, however, that deletion of some genes is embryonically lethal and hence they cannot be studied as adults. Moreover, the permanent deletion of a gene throughout life may result in compensatory mechanisms becoming established that would not normally be present. An interesting example is the oxytocin knockout mouse. Despite the clearly established role of oxytocin in parturition, these mice give birth normally, suggesting that in the absence of oxytocin, some other mechanism has compensated for this critical reproductive function.

3.6.3. CONDITIONAL KNOCKOUTS

The next generation of transgenic models provide the ability to undertake tissue-specific deletion of genes, potentially in a time-dependent manner, using conditional transgenics. Cre-*loxP* technology has been described as “the first choice for conditional gene deletion experiment.” The site-specific bacterial recombinase, Cre, directs recombination between *loxP* (locus of crossover) sites, resulting in deletion of DNA sequences between two *loxP* sites. This characteristic has been used to generate transgenic mice in which deletion of genetic material can be achieved in selected cells at a specific time in development. The most simple application requires generation of two lines of transgenic mice: one in which *loxP* sites have been incorporated around a critical region of a gene of interest (the allele is said to be “floxed”), and a second in which Cre recombinase is expressed only in those cells in which deletion is required. The floxed mice are generated using targeting vectors in embryonic stem cells and require homologous recombination to insert in the correct location. Such mice can be bred to homozygosity, and because the *loxP* sites are placed in introns, expression of the gene should continue to be normal. The cell-specific expression of Cre is achieved using cell-specific gene promoters driving Cre in transgenic mice generated by pronuclear oocyte injections. Promoter design allows time- and/or tissue-specific expression of Cre. Crossing the Cre mouse with the mice containing the floxed gene of interest will result in the floxed gene being permanently deleted in all cells expressing Cre, with subsequent loss of function of that gene in that tissue. Research using such mice is now being published. For example, it has been possible to specifically delete the estrogen receptor from neurons only, demonstrating that negative feedback regulation of LH secretion

by estrogen is mediated through neurons (rather than at the level of the pituitary gland).

3.7. Case Study in Application of Transgenic Methodologies to Investigate Hypothalamic Function

The following case study will serve as an example of the potential of applying these innovative research methodologies to long-standing questions in hypothalamic function (see Journal of Neuroendocrinology, Volume 19, page 561, 2007). Mapping neuronal circuitry within the hypothalamus is one of the most powerful tools in understanding how specific cell populations are regulated. The GnRH neurons represent an extremely difficult population of neurons to study, because they are scattered over a wide area throughout the hypothalamus. Traditional tract tracing approaches, as described above, were not particularly useful, as they could only identify fibers passing into the region of GnRH neurons and could not identify whether those fibers actually innervated GnRH neurons. What was required was a specific tract tracing method that only labeled neurons going to GnRH neurons. This has recently been achieved using a conditional viral tract tracing method. A genetically modified pseudorabies virus was constructed that could only replicate (and therefore infect other cells) in the presence of Cre recombinase. Pseudorabies has previously been used as a retrograde tracer, with the ability to infect neuronal networks by crossing from one neuron to another through synaptic connections. In the current study, the modified pseudorabies virus was injected into the region containing GnRH neurons of a transgenic mouse that was expressing Cre under the control of the GnRH promoter. Presumably, the virus would have been taken up into many cells in this area, but only in GnRH neurons was the virus activated by the presence of Cre. Having been activated, the virus proceeded to infect all neurons that had direct afferent synaptic connections on the GnRH neurons. After a subsequent round of replication, the virus would then infect the second-order neurons that innervate the primary afferents of GnRH neurons, and so on. By carefully tracking the spread of the virus across time, investigators were able to map the entire network of neurons that influence GnRH neurons. By combining this innovative method with other more classical approaches, such as detecting expression of estrogen receptors, or detecting activation of neurons by Fos labeling, it will be possible to define patterns of hormonal action that might indirectly influence the GnRH neurons.

4. PHYSIOLOGIC PROCESSES CONTROLLED BY THE HYPOTHALAMUS

4.1. The Hypothalamus Regulates Pituitary Hormone Secretion

4.1.1. GENERAL CONCEPTS

4.1.1.1. Characteristics of a Neuroendocrine System. As noted earlier, the key feature of a neuroendocrine system is the existence of the neurohemal area; the external zone of the median eminence, at which neurosecretory axon terminals converge upon a capillary bed that ultimately leads to and affects the secretions of the adenohypophysis. Similarly, neurosecretory axons comprising the hypothalamo-hypophyseal tract terminate on sinusoids in the neurohypophysis and ultimately secrete their products to the peripheral circulation to affect visceral processes. The axons terminating in the external zone of the median eminence secrete release and release-inhibiting hormones into the hypothalamo-hypophyseal portal plasma. In order to meet the definition of release or release-inhibiting hormones, the substances in portal plasma must fulfill certain criteria (Table 16): (1) they must be *extractable* from hypothalamic or median eminence tissue; (2) they must be present in hypophyseal portal blood in *greater amounts* than in the systemic circulation; (3) varying concentrations of a particular release or release-inhibiting hormone in portal plasma must be *correlated with* varying secretion rates of one (or more) of the anterior pituitary hormones in a variety of experimental conditions; (4) the suspected hormone should stimulate or inhibit one or more pituitary hormone(s) when administered *in vivo* or applied to pituitary cells or tissues *in vitro*; (5) *inhibitors* that antagonize the actions of the release or release-inhibiting hormones should block or stimulate anterior pituitary hormone secretion;

Table 16
Required Characteristics of Neurohormones

1. Activity must be extractable from whole hypothalamus or median eminence tissue.
2. Concentration in hypophyseal portal blood must be greater than systemic circulation.
3. Dynamics in portal plasma must be correlated with dynamics of adenohypophyseal hormone secretion.
4. Extracted material must be active *in vivo* and *in vitro*.
5. Inhibitors of neurohormones should affect physiologic end point.
6. Target cells should have receptors for neurohormones.

and (6) target cells should have *receptors* for the release or release-inhibiting hormones.

4.1.1.2. Concept of Feedback. The hypothalamo-pituitary-target axes can be characterized as a set of links over which information flows. As information is transmitted from link to link, not only does it stimulate or depress a biological response in the next link, but it also influences the activity of an earlier link. Such an influence is referred to as a *feedback*. In general, there are two types of feedbacks. A *negative feedback* (Fig. 25) is one in which the activity of the downstream link inhibits the activity of one or more upstream links. Conceptually, regulation of room temperature by a thermostatically driven furnace fits this model. The furnace, in response to regulation by a thermostat, raises the temperature of the room to a point preset on the thermostat. Once that temperature is achieved, the thermostat senses that level and turns off the furnace. If the thermostat is inoperative, the furnace runs extensively and the temperature in the room rises to the limits of the furnace. In neuroendocrinology, an example of a negative feedback is the ability of adrenal corticosterone to inhibit CRH secretion into portal blood. A *positive feedback* is one in which the downstream link enhances the activity of one or more upstream links. Conceptually, this is a more difficult mechanism to describe accurately. A voice-activated recording device is perhaps the best example of a positive feedback. The voice begins the recording device. When the voice ceases, the recording device stops. Unlike negative feedback, which is a dampening process and long-term, positive feedback is relatively brief and inherently unstable. The feedback signal can be provided by a hormone itself or by a nonhumoral metabolite. The classic example of a positive feedback in neuroendocrinology is the ability of ovarian estradiol to stimulate GnRH secretion into portal blood (described later). Feedbacks can take several routes (Fig. 26). In the case of the hypothalamo-pituitary-target gland axis, a *long loop feedback* would be blood-borne from the peripheral target gland to affect the hypothalamus or pituitary. A *short loop feedback* might be exemplified by a pituitary hormone influencing the secretion of its hypothalamic release or release-inhibiting factor. Finally, an *ultrashort loop feedback* is represented by a pituitary hormone effecting its own secretion through an autocrine mechanism.

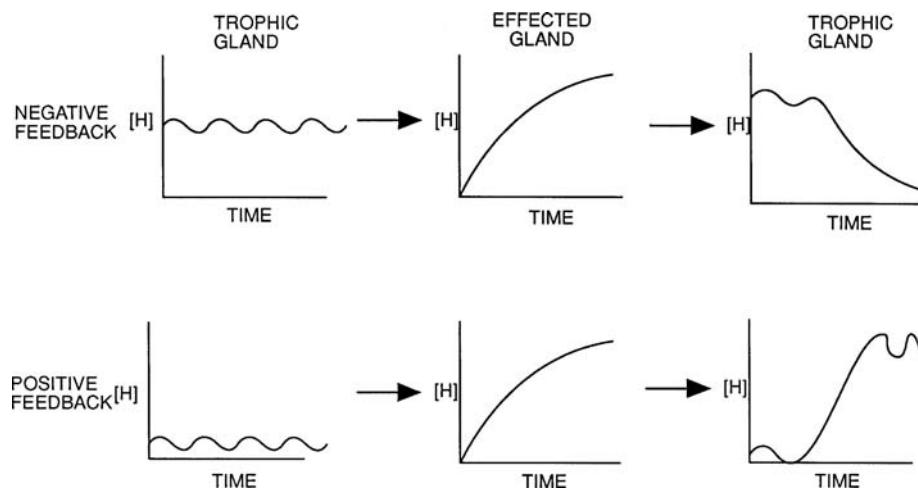


Fig. 25. Diagrammatic representation of a negative feedback (*upper sequence*) and positive feedback (*lower sequence*) in the endocrine system. In a negative-feedback system, the tropic gland (such as the adenohypophysis) secretes a signal (such as TSH) that stimulates the target gland (the thyroid) to secrete its product (thyroid hormone), which in turn feeds back to inhibit TSH secretion. In a positive-feedback system, the tropic gland (the adenohypophysis) secretes a signal at low rates (such as LH) that stimulates the target gland (the ovary) to secrete its product (estradiol) whose increasing secretion rate allows the tropic gland to secrete LH in larger amounts. [H] = hormone concentration in blood.

4.1.2. REGULATION OF THE ADENOHYPOPHYSIS BY THE HYPOTHALAMUS

4.1.2.1. Gonadotrophs. The overwhelming evidence favors the concept that the hypothalamic decapeptide known as luteinizing hormone-releasing hormone (LHRH; also known as GnRH) is the peptide in hypophyseal portal blood that is the physiologic humoral stimulator of LH and FSH secretion. Though FSH-releasing activities devoid of LH-releasing activity have been isolated from the hypothalamus, a distinct FSH-releasing hormone has yet to be identified. GnRH regulates LH and FSH secretion from the gonadotrophs of the adenohypophysis in both basal and ovulation-inducing “surge” states in female mammals. In rodents, the “surge” center is the medial preoptic area, whereas the basal center is in the medial basal hypothalamus. Males lack a functional “surge” center. The ovarian steroids, estrogen and progesterone, inhibit LH and FSH secretion by acting directly at the gonadotroph and also at the medial basal hypothalamus to inhibit GnRH release into portal blood. In addition to this negative feedback action, the ovarian steroids can also stimulate a preovulatory surge of LH and FSH secretion. Prolonged elevations in estrogen from the developing follicle induce a switch from negative to positive feedback, acting at the medial preoptic area to stimulate a surge of GnRH into portal blood. This

is thought to be mediated through a noradrenergic mechanism. In rodents, surgical isolation of the medial preoptic area from the medial basal hypothalamus will prevent a steroid-induced surge of LH secretion. Neither ovarian or testicular steroids will induce an LH surge in males. But the gonadal steroids will inhibit LH and FSH secretion regardless of the sex of the recipient. In primates, there is evidence that the surge center may reside in the basal hypothalamus and that the ovarian steroid merely sensitizes the gonadotrophs to respond to unvarying pulses of GnRH. Monkeys bearing surgically isolated medial basal hypothalami and infused with pulsatile GnRH respond to estradiol with an LH surge. This has led to the concept that the medial basal hypothalamus is a *pulse generator* for GnRH release into portal blood. There is a clear sexual dimorphism of the medial preoptic area: males have a more intensely stained medial preoptic nucleus within this area than do females. Such differentiation occurs perinatally. In rodents, castration of males within the first days of life prevents the appearance of this sexually dimorphic area in adults. Moreover, when adult, neonatally castrated males can respond to an estradiol challenge with a preovulatory-like LH surge. If testosterone is administered to phenotypic female rodents within the first days of life, they develop the male-type sexual dimorphic nucleus and the male pattern of gonadotropin secretion. Because they

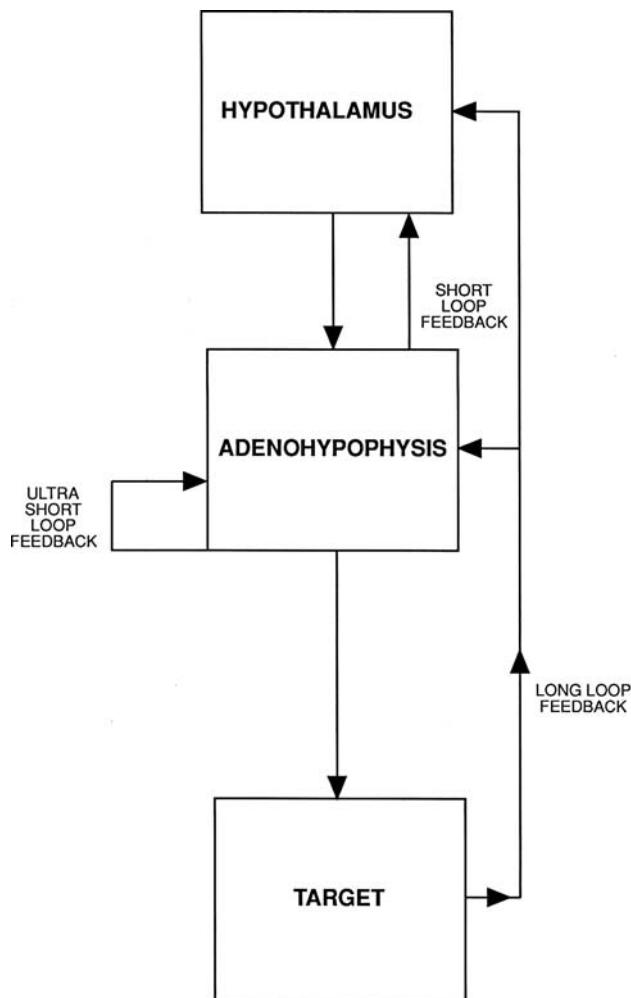


Fig. 26. Diagrammatic representation of feedback loops. In the hypothalamo-pituitary-target gland axis, a long loop feedback would be blood-borne from the peripheral target gland to effect the hypothalamus or pituitary. A short loop feedback could be exemplified by a pituitary hormone influencing the secretion of its hypothalamic release or release-inhibiting hormone. An ultrashort loop feedback is characterized by a pituitary hormone effecting its own secretion through an autocrine mechanism.

lack a surge center, they are anovulatory. Though GnRH is the physiologic regulator of LH and FSH secretion, other neuropeptides subserve a similar function, either as neurohormones, neurotransmitters, or neuromodulators. These are listed in Table 17.

4.1.2.2. Lactotrophs. As noted earlier, the dominant hypothalamic control over pituitary prolactin secretion is inhibitory. Removal of hypothalamic influence over the adenohypophysis results in enhanced secretion of prolactin. Thus, stalk transection, destruction of the medial basal hypothalamus, or placement of pituitary fragments or cells in culture results in hypersecretion of prolactin. Moreover, *in vivo* treatment with dopamine antagonists results in

hypersecretion of prolactin. Conversely, *in vivo* treatment with dopamine agonists depresses pituitary prolactin secretion. These data, coupled with the inverse relationship between dopamine levels in portal blood and peripheral blood levels of prolactin suggest that dopamine is the prolactin release-inhibiting hormone. However, recent studies of the dynamics of prolactin release in response to lowered dopaminergic tone suggest that the lactotroph must also be under the influence of prolactin-releasing hormones (PRH) of hypothalamic origin. Though thyrotropin-releasing hormone (TRH) is one of the most widely studied candidates, others (listed in Table 17) have prolactin-releasing properties and may also play a role. Prolactin secretion in response to exteroceptive stimuli such as suckling thus may involve not only a lowering of

Table 17
Peptide and Amines That Act Directly
on Adenohypophyseal Cells

Cell type	Peptide or amine	Other peptides or amines
Gonadotroph	GnRH	VIP CCK NPY Substance P Galanin Neurotensin
Lactotroph	Dopamine*	TRH Oxytocin VIP Angiotensin II Somatostatin GnRH
Thyrotroph	TRH	Somatostatin*
Corticotroph	CRH	AVP
Somatotroph	GHRH	TRH
		Somatostatin*

* = inhibit function of cell.

dopamine levels in portal blood but also an increase in the portal blood concentration of a putative PRH.

4.1.2.3. Thyrotrophs. There is little doubt that the tripeptide pyro glu-his-pro-NH₂ is *the* thyrotropin-releasing hormone (TRH; Table 17). TRH is the physiologic stimulator of TSH secretion from the adenohypophysis. The cell bodies whose axons terminate upon the external zone of the median eminence are found primarily in the paraventricular nucleus. TSH, in turn, stimulates thyroid hormone (thyroxine and triiodothyronine) secretion from the thyroid gland. The thyroid hormones, in turn, diminish the release of TSH by lowering the response of the thyrotroph to TRH. This is a classic negative feedback control system. Removal of the thyroid gland enhances the release of TSH into the peripheral circulation without affecting portal blood levels of TRH. This further suggests that primary control of TSH secretion by thyroid hormones does *not* reside at the hypothalamus. The hypothalamus provides the drive (TRH), but the thyroid gland negatively regulates (by thyroid hormone) the response of the thyrotroph (TSH) to that drive.

4.1.2.4. Corticotroph. ACTH secretion from the corticotroph is controlled primarily by corticotropin-

releasing hormone (CRH) released into portal blood. ACTH stimulates the release of the steroid hormones of the adrenal cortex, which in turn feeds back negatively to inhibit ACTH release by acting at the hypothalamus as well as the adenohypophysis. It is now apparent that arginine vasopressin (AVP) is also a potent ACTH-releasing hormone. Portal blood levels of both AVP and CRH are positively correlated with ACTH-releasing stimuli such as stress. Thus, ACTH release is not due to the action of a single peptide but is the result of the actions of a hypothalamic *complex*.

4.1.2.5. Somatotroph. Though the somatotroph is predominately under the stimulatory influence of hypothalamic growth hormone-releasing hormone (GHRH), it is also under the opposing influence of a growth hormone release-inhibiting hormone, somatostatin (SS). Not only does each neurohumoral peptide affect growth hormone (GH) secretion through distinct receptor sites on the somatotroph, but each plays a reciprocal neuromodulatory role on the other. The SS neurons also directly innervate GHRH neurons with the result of diminishing GHRH release into portal blood and consequently reducing GH release into the peripheral circulation. Conversely, stimuli known to release GH also suppress release of SS into portal plasma. Feedback control of GH secretion does not completely fit the models of classic negative or positive feedback by target endocrine organs. Classic long loop feedback may occur, through GH-induced secretion of insulin-like growth factor-1 (IGF-1; formerly known as somatomedin-C) from the liver. IGF-1 mediates a number of GH actions in the body, particularly on skeletal muscle and bone. IGF-1 receptors are found in the hypothalamus, although there is little evidence for regulation of GHRH or SS by IGF. GH appears to exert short loop negative feedback control over its own secretion, predominately mediated through GH receptors on SS neurons in the periventricular nucleus, and indirectly, through actions of SS on GHRH neurons. But in addition, other factors exert important feedback regulation over GH secretion. For example, one action of GH is to mobilize glucose into the blood. Hypoglycemia will stimulate GH secretion by stimulating the release of GHRH into portal blood. Conversely, hyperglycemia will inhibit GH secretion by increasing SS and decreasing GHRH levels in portal blood.

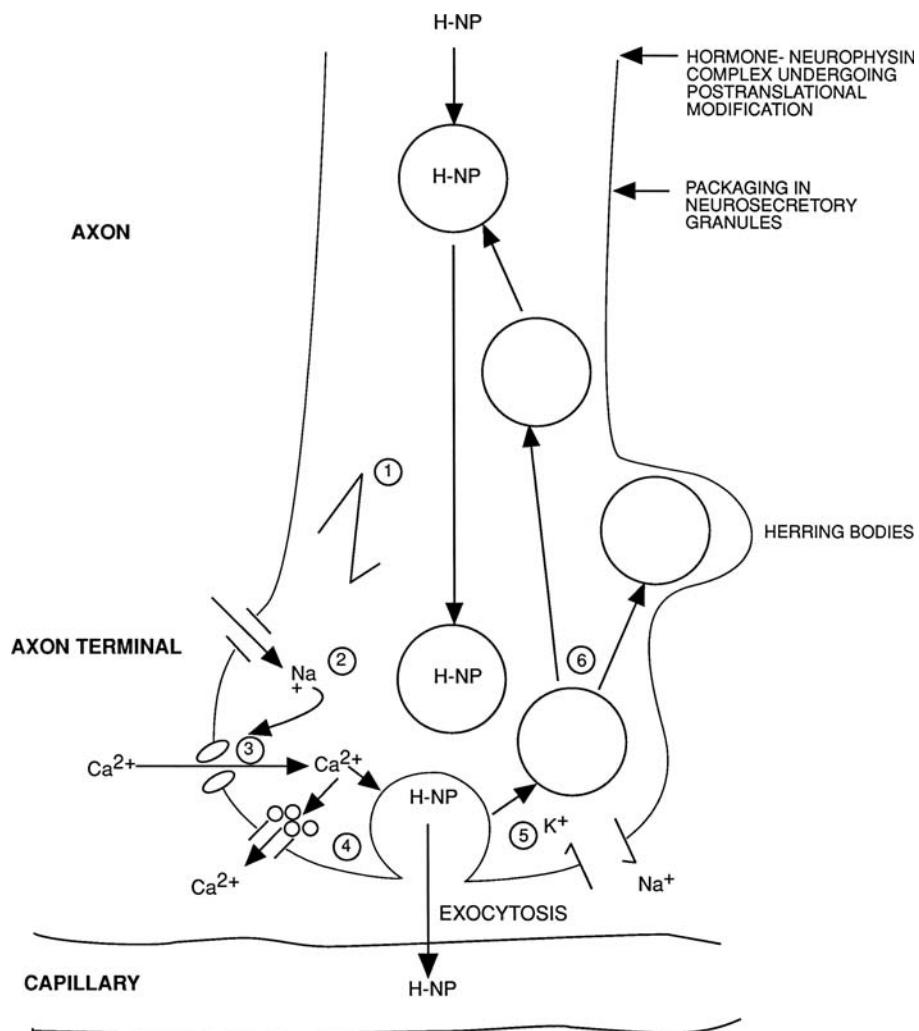


Fig. 27. The mechanism by which the neurohypophyseal peptide-neurophysin complex is axonally transported, processed, packaged, and secreted from the axon terminal. As the hormone neurophysin complex is transported down the axons in the hypothalamo-hypophyseal tract, further posttranslational processing is taking place. The mature complex is then packaged into neurosecretory granules whose arrival at the axon terminal coincides with the arrival of the action potential (1). The membrane of the granule fuses with the axon terminal membrane and the product is exocytosed. The action potential is believed to play a role in the process by causing depolarization and entry of sodium (2), which in turn allows entry of calcium through specific channels (3). Calcium plays an incompletely understood role in the exocytic process. The intracellular calcium is then packaged into microvesicles (4) and extruded, and the membrane potential is restored by a sodium-potassium pump (5). The membranes of the evacuated neurosecretory granules are reformed (6) and either packaged into lysosomes and degraded or recycled in areas of nonterminal swelling known as Herring bodies.

4.1.3. THE HYPOTHALAMUS AND NEUROHYPOPHYSEAL FUNCTION

4.1.3.1. Mechanism of Secretion of Neurohypophyseal Hormones. The hormone-neurophysin complex (vasopressin-neurophysin II or oxytocin-neurophysin I) are synthesized in cell bodies of the supraoptic or paraventricular nuclei. The complexes, still undergoing posttranslational processing, are transported down the long axons composing the hypothalamo-hypophyseal tract to terminals adjacent

to fenestrated capillaries in the neurohypophysis (Fig. 27). Adjacent to the fenestrated capillaries in the neurohypophysis are specialized glial-like cells known as *pituicytes*, which regulate the microenvironment of the terminals. During this voyage, they are packaged in neurosecretory granules. Once at the axon terminal, the granule membrane fuses with the membrane of the axon terminal, and the hormone-neurophysin complex is exocytosed. The process coincides with the arrival of the action potential, which depolarizes the membrane allowing entry of

sodium ions. These, in turn, permit the opening of calcium channels, which plays an incompletely understood role in the exocytotic process. After exocytosis of the neurohormone, intracellular calcium is packaged into microvesicles and extruded, and the membrane potential is restored by a sodium-potassium pump. The membranes of the evacuated neurosecretory granules are reformed from the surface of the axon terminal where they are either packaged into lysosomes and degraded or recycled; usually in areas of nonterminal swelling known as Herring bodies.

4.1.3.2. Stimuli for Secretion of Vasopressin.

The two main drives for vasopressin secretion are an increase in osmolality of the plasma and a decrease in plasma volume (Table 18). These can be either interrelated or independent stimuli. Water deprivation causes an increase in plasma osmolality and a diminution of intracellular water. A change in plasma osmolality of as little as 1% is detected by osmoreceptive neurons, which are distinct from the vasopressin magnocellular neurons in the hypothalamus. The osmoreceptive neurons stimulate vasopressin synthesis and release from the magnocellular neurons in the supraoptic area at a threshold of 280 mOsm/kg. The osmoreceptor neurons can also stimulate thirst but with a higher threshold of 290 mOsm/kg. Vasopressin release is also stimulated by a 5% to 10% decrease in blood volume, blood pressure, or cardiac output. Hypovolemia is perceived by pressure receptors in the carotid and aortic arch as well as stretch receptors in the walls of the left atrium, pulmonary veins, and the juxtaglomerular apparatus of the kidney. The afferent impulses of these sensors are carried via the ninth and tenth cranial nerves to the medulla

and then through the midbrain over noradrenergic synapses to the magnocellular vasopressinergic neurons of the supraoptic nucleus. In the absence of any change in pressure, the receptors tonically inhibit vasopressin secretion. With acute volume depletion such as that caused by hemorrhage, noradrenergic inhibitory tone from the medulla to the hypothalamus is decreased resulting in an increase in secretion of vasopressin. Volume depletion also stimulates central renin-dependent angiotensin release, which also stimulates vasopressin secretion and thirst.

4.1.3.3. Stimuli for Secretion of Oxytocin.

Suckling is the best described stimulus for oxytocin secretion (Table 18). As one might expect, the pathways are similar to that of vasopressin. The suckling stimulus is carried over afferent spinal pathways to the medulla and midbrain and then through cholinergic synapses to the paraventricular nucleus. Oxytocin release is pulsatile. Nipple suction by the young leads to synchronized activation of action potentials for 2 to 4 s in the paraventricular nucleus. From a resting “spontaneous” background of 1 to 10 spikes/s, these neurons generate a synchronized series of 70 to 80 action potentials within 3 to 4 s of application of the stimulus resulting in the secretion of 0.5 to 1.0 mIU of oxytocin. This is followed by milk ejection from the mammary gland by 12 to 15 s later. These pulses of neuronal activity occur uniformly every 4 to 8 min. This characteristic of periodic bursting of action potentials at high frequency appears to be important for oxytocin secretion and consequent milk ejection. The stimuli for oxytocin release during labor appear to be multiple. Not only is activation of cervico-vaginal stretch receptors by the growing conceptus an

Table 18
Physiologic Inputs for Stimulation and Inhibition of Vasopressin and Oxytocin Secretion

Hormone	Stimulation	Inhibition
Vasopressin	↑ plasma osmolality ↓ plasma volume ↓ blood pressure ↓ cardiac output (α↑, β↓) noradrenergic tone	↓ plasma osmolality ↑ plasma volume ↑ blood pressure ↑ cardiac output
Oxytocin	Suckling ↑ activation of cervicovaginal stretch receptors ↓ placental progesterone ↑ fetal cholesterol ↑ sensory stimulation	Stress

↑ = increase.

↓ = decrease.

Table 19
Control of Cardiovascular Function by the Hypothalamus

Area	Response
Lateral and posterior hypothalamus	↑ arterial pressure ↑ heart rate
Preoptic area	↓ arterial pressure ↓ heart rate

↑ = increase.

↓ = decrease.

important signal, but also a hormonal background of diminishing placental progesterone and elevated fetal free cortisol act in concert to stimulate oxytocin secretion sufficient to enhance contractions of the uterus. Interestingly, oxytocin secretion has a significant sensory component. In the human female, merely playing with the infant or sensing the cries of a hungry infant will cause milk ejection. In contrast, emotional stress will inhibit the secretion of oxytocin.

4.2. The Hypothalamus Regulates Autonomic Processes

4.2.1. CARDIOVASCULAR FUNCTION

Independent of the control of blood pressure through the neuroendocrine function of the hypothalamus, the cardiovascular system is influenced by the hypothalamus through the autonomic nervous system (Table 19). These effects are mediated primarily by the sympathetic system through the vagus nerve. Stimulation of the posterior and lateral hypothalamus increases arterial pressure and heart rate, whereas stimulation of the preoptic area decreases heart rate and arterial pressure. These effects are mediated by the cardiovascular centers in the medulla and pons. Cardiovascular regulation in response to alterations in environmental temperature or defense reactions is also mediated by the hypothalamus. In response to a hot environment, dilation of blood vessels of the skin and constriction of deep visceral vessels occur, whereas cold exposure induces the converse responses. These are controlled by the preoptic/anterior hypothalamic areas. The defense reaction that is characterized by cutaneous vasoconstriction and muscular vasodilation is the consequence of discharge of sympathetic cholinergic vasodilators as well as sympathetic adrenergic excitation. These effects can be induced by selective stimulation of the anterior and posterior hypothalamus.

4.2.2. THERMOREGULATORY FUNCTION

The control of body temperature by the hypothalamus is a classic example of an *integrative* approach to alteration of the *internal milieu*. The hypothalamus oversees *autonomic compensations* such as alterations of blood flow and sweating, *endocrine compensations* such as metabolism-regulating alterations of thyroid function, and *musculoskeletal compensations* such as shivering, panting, and piloerection (Table 20). Temperature regulation by the hypothalamus is also a nonendocrine example of a feedback mechanism. The regulatory system actually collects temperature information from two sources: peripheral sources such as the skin, visceral structures, and spinal cord and central sources such as thermosensors in the preoptic area/anterior hypothalamus whose neurons are activated or inactivated by the temperature of the blood bathing them. The hypothalamus bears dual mechanisms for controlling heat dissipation and heat conservation. The heat dissipation centers lie in the preoptic area/anterior hypothalamus, and the heat conservation centers lie in the posterior hypothalamus. Electrical stimulation of the preoptic area/anterior hypothalamus favors dilation of cutaneous blood vessels, panting, and suppression of shivering. All result in a drop in body temperature. Conversely, electrical stimulation of the posterior hypothalamus leads to cutaneous vasoconstriction, visceral vasodilation, shivering, and a suppression of panting. The metabolic response to temperature alteration also involves the hypothalamus. Exposure to cold enhances the animal's heat-generating metabolic rate by stimulating TRH-activated TSH secretion and subsequent thyroid hormone secretion. It is clear from recordings of neurons in the preoptic area/anterior hypothalamus that thermosensitive neurons are of two separate types: warm-sensitive and cold-sensitive. Thus, warming of either the skin or hypothalamus results in enhanced firing of warm-sensitive neurons and decreased firing of cold-

Table 20
Thermoregulatory Function of the Hypothalamus

<i>Compensation</i>	<i>Area</i>	<i>Response</i>
Autonomic	Preoptic area	Dilation of cutaneous blood vessels sweating
	Posterior hypothalamus	Vasoconstriction
Musculoskeletal	Preoptic area	Panting
	Posterior hypothalamus	Suppression of shivering Shivering
Endocrine	Preoptic area	Suppression of panting Piloerection Thyroid function

sensitive neurons. Conversely, cooling of the skin or hypothalamus leads to opposite effects. Thus, these neurons serve to integrate information from the periphery as well as the central nervous system. Interestingly, the hypothalamus coordinates voluntary behavioral adjustments to extremes in environmental temperatures sensed at both the hypothalamus and the skin. For example, in both rats and monkeys trained to make behavioral adjustments to a hot environment, local warming of the hypothalamus in the face of normal ambient temperature results in the appropriate behavioral adjustment to warmth. The hypothalamus will also integrate a summation of the responses. Thus, when both the hypothalamus and the environment are warmed, the behavioral response is greater than either alone. In a hot environment, cooling of the hypothalamus will completely suppress the behavioral adjustment to elevation of environmental temperature. Thus the hypothalamus assumes supremacy in the behavioral responses to alteration in temperature. Finally, the hypothalamus mediates the response to pyrogens in pathologic states. Body temperature is regulated around a set-point. Substances that allow the temperature to deviate from that set-point, *pyrogens*, can be produced by macrophages in disease states. The preoptic area appears to respond to one such pyrogen,

interleukin-1. It has been suggested that the prostaglandins mediate the response to certain pyrogens and act at the preoptic area. Antipyretics such as indomethacin may act by blocking the synthesis of prostaglandins. The brain also contains a nearby *antipyretic area* within the septal nuclei. This area may use the peptide vasopressin. Injection of vasopressin directly into this area counteracts the effects of many known pyrogens. Thus, antipyretics may also act by stimulating the release of vasopressin. Injection of a vasopressin antagonist prevents the antipyretic effects of indomethacin.

4.2.3. DEFENSIVE FUNCTION

The hypothalamus is also responsible for the preparation of the organism to respond to threatening or stressful situations. The so-called *flight-or-fight* response actually represents an integrated constellation of responses to prepare for stressful situations (Table 21). Many of these responses are directly controlled by the hypothalamus, whereas others are indirectly controlled by the hypothalamus through its control of the endocrine system. The hypothalamus stimulates a variety of cardiovascular compensations. In response to a perceived threat, blood pressure, heart rate, force of contraction, and rate of cardiac conduction velocity increase. The rate and depth of

Table 21
Components of the Flight-or-Fight Response

- | | |
|-----------------------------------|---|
| 1. Increase in: | Blood pressure, heart rate, force of contraction, rate of conduction velocity. |
| 2. Increase in: | Rate and depth of respiration. |
| 3. Shift in: | Blood flow from skin and splanchnic organs to skeletal muscles, heart, brain. |
| 4. Metabolic adjustments: | Enhanced glycogenolysis and lipolysis. |
| 5. "Other" autonomic adjustments: | Mydriasis, accommodation for far vision, contraction of spleen capsule, piloerection, inhibition of gastric motility and secretion, contraction of gastrointestinal sphincters, sweating. |

respiration increases. There is a shift of blood flow from the skin and splanchnic organs to the skeletal muscles, heart, and brain. Metabolic adjustments are made in anticipation of increased energy requirements. These are enhanced glycogenolysis and lipolysis. In addition to the cardiovascular adjustments, there are other autonomic alterations. These would be mydriasis, ocular accommodation for far vision, contraction of the spleen capsule leading to increased hematocrit, piloerection, inhibition of gastric motility and secretion, contraction of gastrointestinal sphincters, and sweating. Some of these are regulated by the autonomic nervous system directly, whereas others are controlled by hormones secreted in response to stressful stimuli. Classically, epinephrine is secreted from the adrenal medulla in response to acute stressors. This catecholamine controls many of the metabolic demands of the flight or fight response. Additionally, glucocorticoids are secreted from the adrenal cortex in response to stressful stimuli. Secretion of glucocorticoids are controlled by ACTH secreted from the pituitary gland under the influence of hypothalamic CRH and AVP. In long-term stressful situations, this leads to suppression of the immune system. Other hormones whose secretion is stimulated in response to stress (and their putative roles in stress responses) are β -endorphin (pain perception), vasopressin (renal function), glucagon (carbohydrate mobilization), and prolactin (immune responses). Growth

hormone and insulin are typically inhibited during stressful circumstances. Many of these autonomic and hormonal responses are controlled by the anterior and ventromedial hypothalamus.

4.3. Regulation of Behavioral Processes

4.3.1. INGESTIVE BEHAVIOR

4.3.1.1. Hypothalamic Control of Feeding Behavior. The role of the hypothalamus is to coordinate ingestion with parallel neuroendocrine responses and long-term regulation of metabolism and adiposity. Ingestion during short-term meals is coordinated by brain-stem sensory and motor circuits. The nucleus of the solitary tract (NST) relays gustatory and visceral information about ingested food during individual meals by direct and indirect pathways to the hypothalamus. A number of peripheral peptides have been identified that act before and during meals to influence appetite and satiety acutely, including the orexigenic hormone ghrelin (released from the stomach), and the satiating hormones amylin (released from the pancreas) and cholecystokinin (CCK), peptide tyrosine-tyrosine 3-36 (PYY3-36), and glucagon-like peptide-1 (GLP-1), the last three released from the intestine. These peptides may act on vagal and brain-stem circuits (CCK, amylin) or act directly on hypothalamic neurons (ghrelin, PYY3-36, GLP-1).

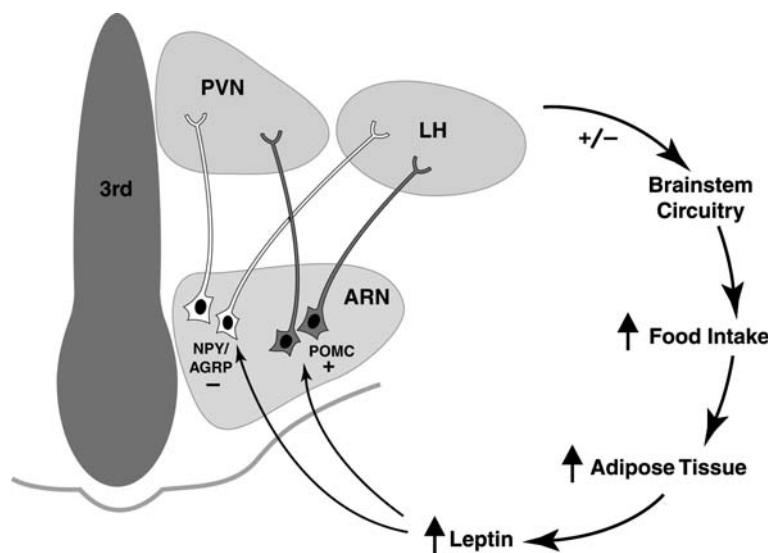


Fig. 28. Hypothalamic integration of ingestive behavior and adiposity. The acute behavior of food intake is largely regulated by neural networks of the pons and medulla. Increased food intake leads to increased fat mass and elevated leptin levels. Leptin provides negative feedback to the hypothalamus to decrease feeding, primarily by decreasing synthesis and release of NPY/AGRP (orexigenic peptides) and increasing synthesis of POMC and hence α MSH (an anorexic peptide) in neurons of the arcuate nucleus (ARN). The ARN neurons project to the paraventricular nucleus (PVN) and the lateral hypothalamus (LH), which in turn modulate brain-stem circuitry to decrease food intake and maintain a stable level of adiposity. Food deprivation and reduced fat mass has the opposite effect (to increase food intake) by reducing negative feedback by leptin.

To achieve long-term weight regulation, the appetitive systems of the hypothalamus must also monitor energy storage (in peripheral adiposity) and generate neural signals back to the brain stem to increase or decrease food intake. Body weight is regulated by the balance of energy expenditure (e.g., basal metabolism and locomotor activity) and food intake. Total food intake is the product of number of meals (meal frequency) and meal size. Therefore, body weight can be altered through changes in hunger or appetite (i.e., to change meal initiation and frequency) or sensitivity to satiety signals (i.e., to alter meal size). The regulation of body weight and food intake is achieved by a complex network in the hypothalamus involving multiple transmitter and neuropeptide systems (Fig. 28).

4.3.1.2. Leptin Is a Negative Feedback Adiposity Signal. In order to regulate body weight and long-term energy balance, the hypothalamus must monitor the amount of long-term energy storage in the fat. Although the hypothalamus is sensitive to several other adiposity signals such as insulin, the adipose hormone leptin serves as the primary negative feedback signal to the brain to regulate fat mass. Leptin is a 127 amino acid peptide secreted into the circulation from adipocytes; plasma leptin levels are proportional to total body adiposity. Weight loss and food deprivation, which rapidly decrease fat mass and the metabolic rate of adipocytes, lead to a rapid decrease in plasma leptin. Overfeeding, refeeding after fasting, or increased adipose tissue mass increases plasma leptin. Because leptin is a negative feedback signal, increased leptin secreted by increased fat will reduce food intake; decreased leptin during weight loss causes increased appetite to drive compensatory hyperphagia. Exogenous systemic or central administration of leptin in animals reverses many of the physiologic and behavioral correlates of fasting. In normally feeding or obese animals, leptin administration decreases appetite, food intake, and increases metabolic rate, resulting in weight loss. The leptin system is functional in humans, because a mutation in the leptin gene that blocks leptin synthesis, or a mutation in the leptin receptor that results in functional hypoleptinemia, causes profound obesity and other neuroendocrine deficits. In these mutants without leptin signaling, the hypothalamus responds as if the body has no fat reserve of energy: in order to compensate for the apparent starvation state, profound hunger and overeating occurs.

Leptin enters the ARN and is transported across the blood-brain barrier to act on hypothalamic neurons that express leptin receptors. The leptin receptor is

a member of the cytokine-receptor superfamily, and leptin binding activates JAK-STAT signaling pathways that have both acute effects on neuronal firing rate and long-term effects on gene transcription. Leptin receptors are particularly highly expressed in NPY neurons and POMC neurons of the ARN, in neurons in the dorsomedial VMH, and to a lesser degree on other cell types of the PVN and lateral hypothalamus. The neurons of the ARN compose intermingled but distinct and opposing pathways regulating food intake: the NPY system and the melanocortin system.

4.3.1.3. NPY System. The NPY neurons of the ARN form the major orexigenic or appetite-stimulating system of the hypothalamus. They contain the highest concentration of NPY within the brain; they are also unique in their coexpression of agouti-gene related peptide (AGRP). The primary projection of the NPY neurons is from the ARN to the hypothalamic PVN, but they also have long projections to midbrain, pons, and medulla where they can interact with brain-stem ingestive circuitry directly.

Exogenous NPY administered into the third ventricle or the PVN is the most potent orexigen known: nanogram quantities of NPY acting at Y1 and Y5 receptors cause rodents and primates to eat voraciously for hours. Consistent with the NPY system being a positive signal for food intake, food deprivation and other hunger-inducing treatments cause an increase in NPY mRNA synthesis, peptide synthesis, and NPY release onto the PVN. Furthermore, negative feedback adiposity signals such as leptin and insulin decrease NPY mRNA and peptide levels.

4.3.1.4. Melanocortin System. Intermingled with the NPY neurons of the ARN are POMC neurons, which have projections to the PVN and lateral hypothalamus that parallel the NPY projections. Although POMC serves as a precursor for several neuropeptides (described above), α -melanocyte stimulating hormone (α MSH) is the primary product found in the cells of the ARN. Whereas NPY induces appetite, α MSH from POMC neurons acting on MC4 receptors has an opposing satiating effect. In many ways, POMC neurons respond to adiposity signals with a negative effect to balance NPY's positive effects on food intake. Thus, decreased plasma leptin after food deprivation or weight loss decreases POMC mRNA and peptide levels, whereas increased plasma leptin (e.g., after involuntary overfeeding) increases POMC expression in the arcuate in parallel

with decreased eating. Injection of α MSH or other MC4 agonists into the hypothalamus reduces food intake; antagonism of the MC4 receptor causes increases in food intake. POMC neurons and MC4 receptors are also present in the brain stem, where they may contribute to local ingestive circuitry. The melanocortin system is critical to human physiology, as mutations in POMC or the MC4 receptor cause obesity in humans.

In an intriguing twist, NPY neurons of the ARN also produce AGRP, an endogenous peptide antagonist of the MC4 receptor. Like NPY, AGRP is a potent orexigen, but it acts by postsynaptically antagonizing α MSH signaling from the POMC neurons to reduce satiety and increase food intake. Thus, NPY

neurons and POMC neurons not only have opposing responses to leptin and other adiposity signals, and opposing functional consequences at their target neurons, but also the orexigenic NPY/AGRP neurons directly antagonize the satiating effects of the POMC neurons. Thus, the response of the hypothalamus to peripheral adiposity levels is an adjustment of the balance between NPY/AGRP orexigenic and POMC anorexic systems.

4.3.1.5. MCH and Other Peptide Systems. In recent years, a large number of other peptides within the PVN and lateral hypothalamus have been implicated in the control of feeding and body weight,

Table 22
Peptides Involved in the Hypothalamic Regulation of Ingestion

Peptide	Source	Effect
Leptin	Adipocytes	Anorexic
Val ¹ -Pro-Ile-Gln-Lys-Val-Gln-Asp-Asp-Thr-Lys-Thr-Leu-Ile-Lys-Thr-Ile-Val-Thr-Arg-Ile-Asn-Asp-Ile-Ser-His-Thr-Gln-Ser-Val-Ser-Ser-Lys-Gln-Lys-Val-Thr-Gly-Leu-Asp-Phe-Ile-Pro-Gly-Leu-His-Pro-Ile-Leu-Thr-Leu-Ser-Lys-Met-Asp-Gln-Thr-Leu-Ala-Val-Tyr-Gln-Gln-Ile-Leu-Thr-Ser-Met-Pro-Ser-Arg-Asn-Val-Ile-Gln-Ile-Ser-Asn-Asp-Leu-Glu-Asn-Leu-Arg-Asp-Leu-Leu-His-Val-Leu-Ala-Phe-Ser-Lys-Ser-Cys-His-Leu-Pro-Trp-Ala-Ser-Gly-Leu-Glu-Thr-Leu-Asp-Ser-Leu-Gly-Gly-Val-Leu-Glu-Ala-Ser-Gly-Tyr-Ser-Thr-Glu-Val-Ala-Leu-Ser ¹²⁷		
AGRP	ARN	Orexigenic (α MSH antagonist)
Leu ¹ -Ala-Pro-Met-Glu-Gly-Ile-Arg-Arg-Pro-Asp-Gln-Ala-Leu-Leu-Pro-Glu-Leu-Pro-Gly-Leu-Gly-Leu-Arg-Ala-Pro-Leu-Lys-Thr-Thr-Ala-Glu-Gln-Ala-Glu-Glu-Asp-Leu-Leu-Gln-Glu-Ala-Gln-Ala-Leu-Ala-Glu-Val-Leu-Asp-Leu-Gln-Asp-Arg-Glu-Pro-Arg-Ser-Ser-Arg-Arg-Cys-Val-Arg-Leu-His-Glu-Ser-Cys-Leu-Gly-Gln-Gln-Val-Pro-Val-Val-Asp-Pro-Cys-Ala-Thr-Cys-Tyr-Cys-Arg-Phe-Phe-Asn-Ala-Phe-Cys-Tyr-Cys-Arg-Lys-Leu-Gly-Thr-Ala-Met-Asn-Pro-Cys-Ser-Arg-Thr ¹⁰⁸		
CART	ARN	Anorexic
Gln ¹ -Glu-Asp-Ala-Glu-Leu-Gln-Pro-Arg-Ala-Leu-Asp-Ile-Tyr-Ser-Ala-Val-Asp-Asp-Ala-Ser-His-Glu-Lys-Glu-Leu-Ile-Glu-Ala-Leu-Gln-Glu-Val-Leu-Lys-Lys-Ser-Lys-Arg-Val-Pro-Ile-Tyr-Glu-Lys-Tyr-Gly-Gln-Val-Pro-Met-Cys-Asp-Ala-Gly-Glu-Gln-Cys-Ala-Val-Arg-Lys-Gly-Ala-Arg-Ile-Gly-Lys-Leu-Cys-Asp-Cys-Pro-Arg-Gly-Thr-Ser-Cys-Asn-Ser-Phe-Leu-Leu-Lys-Cys-Leu ⁸⁹		
MCH	LH	Orexigenic
Asp ¹ -Phe-Asp-Met-Leu-Arg-Cys-Met-Leu-Gly-Arg-Val-Tyr-Arg-Pro-Cys-Trp-Gln-Val ¹⁹		
Hypocretin 1/Orexin A	LH	Orexigenic/wakefulness
Gln ¹ -Pro-Leu-Pro-Leu-Cys-Cys-Arg-Gln-Lys-Thr-Cys-Ser-Cys-Arg-Lys-Tyr-Glu-Leu-Leu-His-Gly-Ala-Gly-Asn-His-Ala-Ala-Gly-Ile-Leu-Thr-Leu-Gly ³⁴		
Hypocretin 2/Orexin B	LH	Orexigenic/wakefulness
Arg-Ser-Gly-Pro-Pro-Gly-Leu-Pro-Gly-Arg-Leu-Pro-Arg-Leu-Leu-Pro-Ala-Ser-Gly-Asn-His-Ala-Ala-Gly-Ile-Leu-Thr-Met-Gly ²⁹		

See figures for the sequences of the two major arcuate peptides, orexigenic NPY (Figure 18) and anorexic α MSH (Figure 14). Neurons of the arcuate nucleus (ARN) containing NPY/AGRP or POMC/CART are the major targets of leptin; neurons containing MCH, hypocretin, or orexin, and other peptides such as galanin, CRH, and TRH are targets of the arcuate neurons. There are two peptide products of the hypocretin/orexin gene; note that they may exert an orexigenic effect by regulating wakefulness and arousal rather than appetite.

including melanin-concentrating hormone (MCH), CRH, galanin, oxytocin, hypocretin/orexin, cocaine-and-amphetamine-regulated-transcript (CART), and TRH (Table 22). These other peptide systems are presumed secondary to the major modulatory role of leptin on NPY and POMC neurons of the ARN and can receive input from one or both types of ARN neurons. MCH is a particularly significant member of the secondary systems, because MCH injection into the brain potently induces food intake, and mice lacking MCH are lean with reduced body fat mass.

4.3.1.6. Serotonin and Norepinephrine. As described above, the hypothalamus receives dense innervation of serotonin fibers and norepinephrine fibers from the raphe nuclei and locus coeruleus, respectively, which can modulate the effects of the peptidergic systems. Stimulation of serotonin 5HT-2C receptors reduces food intake by decreasing meal size, and mice lacking 5HT-2C receptors show mild obesity in adulthood. Similarly, norepinephrine release into the PVN acting at beta-2 receptors decreases food intake and causes weight loss. Because the agonists of the monoamines are better characterized and are easier than peptidergic compounds to administer systemically, the serotonin and norepinephrine systems are more accessible targets for the pharmacologic treatment of obesity than are the peptide systems. Thus, the serotonin agonist fenfluramine and the mixed serotonin/norepinephrine reuptake inhibitor sibutramine have been used to decrease food intake and induce weight loss in humans.

4.3.1.7. Obesity and Pharmacologic Control of Appetite. Obesity (defined as a body mass index [i.e., weight divided by height squared] of 30 kg/m^2 or greater) is a growing problem in developed countries. Body fat mass is rapidly increased by easy access to highly palatable, calorie-rich foods. Indeed, by engaging the dopaminergic reward pathways of the limbic system, palatability can override or reset the hypothalamic regulation of body weight. Because it contributes to many other illnesses (e.g., diabetes and cardiovascular disease), obesity is a major public health problem.

In many cases, there may be a genetic contribution to obesity. Mutations that cause obesity or leanness demonstrate the critical role of specific genes in normal behavior and physiology; however, mutations

homologous to obese rodent mutations are exceedingly rare in humans. The genetic predisposition to obesity in some individuals is probably due to more subtle interactions of polymorphisms in multiple appetite-regulating genes.

There are many potential points of body weight regulation that might be targets for therapeutic manipulation. Because of its primary role as an adiposity signal, leptin signaling is an obvious candidate. Similar to the insulin resistance observed in type 2 diabetes, however, human obesity is accompanied by leptin resistance. Plasma levels of leptin are greatly elevated in obese individuals without the expected loss of appetite, and the effects of exogenous leptin on appetite and weight loss are diminished in obese animals. Leptin resistance is thought to occur at the level of the intracellular signaling pathways within cells expressing leptin receptors. Chronic elevation of leptin induces negative feedback via phosphorylation of the leptin receptor at a specific tyrosine residue (Tyr1138), which diminishes leptin receptor activity. Chronic leptin receptor activation also increases transcription of the suppressor of cytokine signaling 3 protein (SOCS-3), which inhibits JAK-STAT signaling downstream of the leptin receptor. The loss of sensitivity to leptin results in increased NPY/AGRP and decreased α MSH tone in the hypothalamus despite high levels of adiposity and circulating leptin. Understanding and reducing leptin resistance in obese individuals is a major goal of current research.

As mentioned above, serotonin and norepinephrine are the most accessible factors; serotonin and norepinephrine receptors are widely distributed in the brain and periphery, however, and thus monoamine treatments are rarely without unwanted side effects. Because the hypothalamus contains unique peptide systems that engage both endogenous appetitive and satiating mechanisms, future treatments may be able to mimic or antagonize these endogenous systems specifically and efficaciously.

4.3.2. HYPOTHALAMIC CONTROL OF DRINKING BEHAVIOR

Thirst is regulated by tissue osmolality and vascular volume. These are controlled, in turn, by AVP secreted from magnocellular neurons in the supraoptic nucleus and also by AII formed in the plasma as well as the brain. Although the drive for water ingestion is through enhanced tissue osmolarity and/or decreased vascular volume sensed by osmoreceptors in the brain and baroreceptors in the brain and periphery, there appears to be a direct effect of hormones

acting at the hypothalamus to mediate the behavioral response. The subfornical organ (SFO) lies near the third ventricle and has fenestrated capillaries permitting entrance of blood-borne materials. The SFO responds to low levels of AII in the blood and conveys information to the hypothalamus. It is possible that the communication is by way of a neuronally-derived AII that affects the preoptic area. In addition, the preoptic area receives information from peripheral baroreceptors. Thus, when water ingestion is required, the baroreceptors and AII stimulate the preoptic area, which in turn activates other areas of the brain to begin drinking. The drive for termination of drinking is less well understood. It is clear, however, that cessation of drinking is not merely the absence of the baroreceptor and osmoreceptor initiating signal.

4.3.3. SEXUAL BEHAVIOR

The circumscribed behaviors leading to pregnancy and propagation of the species depend on the interaction of the gonads and the hypothalamus. In sub-primate mammals, these events are driven by a heightened period of female sexual receptivity, *estrus*, which coincides with the availability of a potentially fertilizable egg in the oviduct. Because these female mammals have reproductive cycles characterized by a heightened receptivity, the cycles are referred to as *estrous cycles* (noun: estrus; adjective: estrous). A similar coincidence of gamete availability with behavioral receptivity is not discretely defined in primates. Because primate cycles are overtly characterized by a period of breakdown of the lining and blood vessels of the uterus, *menses*, these are referred to as *menstrual cycles*. Obviously, sexual receptivity is best

studied in mammals who overtly display the behavior at discrete periods. For this reason, the rat is the most widely studied model of hypothalamic control of sexual behavior.

4.3.3.1. Hypothalamic Control of Sexual Behavior in Females. Sexual receptivity can be quantitated in female rats by a *lordosis quotient*, or *LQ*. Lordosis is the process by which the female arches her back, deflects her tail and stands rigid to allow mounting and intromission by the male. The LQ is the number of times this event takes place divided by the number of attempts at mounting by the male multiplied by 100.

Though the effects of various hormones on sexual behavior are species-specific, the common hormone regulating most sexual behaviors is the ovarian hormone *estrogen* (Table 23). Estrogen receptors are present in the areas of the hypothalamus known to control sexual receptivity. Estrogen secretion is highest when sexual receptivity is increased. Though estrogen, by itself, will enhance sexual receptivity, sexual receptivity is greatest when both estrogen and *progesterone* secretion is highest. Progesterone, by itself, exerts little effect on sexual receptivity. Only in an estrogen-primed animal will progesterone further enhance sexual receptivity. Estrogen acts by stimulating the expression of progesterone receptors in areas of the hypothalamus known to control sexual receptivity. Prolonged exposure to progesterone (as in pregnancy) causes a downregulation of progesterone receptors in the hypothalamus and subsequently a decrease in sexual receptivity.

Table 23
Hypothalamic Control of Sexual Behavior in Females

Chemical mediator	Site of action	Effect
Estrogen	Ventromedial nucleus Midbrain central gray	Increase LQ*
Progesterone	Ventromedial nucleus Preoptic area	Increase LQ response to estrogen
Norepinephrine	Ventromedial nucleus Preoptic area	Modulate progesterone receptors
Acetylcholine	Ventromedial nucleus Medial preoptic area	Modulate LQ response to steroids
Serotonin		Inhibit sexual behavior
GnRH	Midbrain central gray	Heighten response to estradiol
Prolactin	Midbrain central gray	Heighten response to estradiol

LQ = lordosis quotient.

Four parts of the nervous system have been shown to play a role in the control of female sexual behavior: the forebrain, the ventromedial hypothalamic nucleus (VMN), the midbrain central gray (MCG), as well as the lower brain stem and spinal cord. Within the hypothalamus, lesions of the VMN depress sexual behavior in response to estrogen and progesterone. The VMN bears receptors for the ovarian steroids. It is believed that this hypothalamic nucleus modulates the intensity or interpretation of sexually related sensory input. Estrogen receptors are also localized in the MCG. Neurons from both the VMN and spinal cord project to the MCG. The spinal projection transmits tactile information provided by the male's mounting required for the induction of lordosis. The neurotransmitter control of female sexual behavior is well described but cannot be reduced to participation by a single transmitter. Among the catecholamines, ascending noradrenergic fibers from the locus coeruleus regulate lordosis behavior by acting upon α_1 -noradrenergic receptors in the medial preoptic area and VMN. Norepinephrine may act in these areas by modulating progesterone receptors. Dopamine, on the other hand, does not play a role in lordosis behavior but appears to modulate proceptive behaviors such as ear wiggling, hopping, or darting. Acetylcholine plays a role in the facilitation of lordosis behavior by estrogen. Estrogen not only increases the activity of choline acetyltransferase but also increases the activity of acetylcholine receptors in the VMN. Moreover, acetylcholine applied directly to the medial preoptic area or VMN increases lordosis behavior, whereas acetylcholine antagonists applied to these same areas abolish or attenuate lordotic behavior. Among the indolamines, serotonin appears to play an inhibitory role in sexual receptivity. Inhibition of serotonin synthesis excites lordosis behavior, and thus it has been suggested that serotonin is a *sexual satiety* neurotransmitter. A similar role

has been proposed for gamma-aminobutyric acid (GABA).

Some of the hypothalamic peptides that serve a neuroendocrine role in regulating the pituitary gland also serve a neurotransmitter role in regulating sexual behaviors in the female. The best example is GnRH. GnRH applied directly to the hypothalamus of ovariectomized female rats receiving an ineffective dose of estradiol demonstrate lordosis behavior. This suggests that GnRH both stimulates LH secretion and consequent ovulation and subsequent sexual behavior in the female. In addition, not only do preoptic GnRH neurons project to the arcuate nucleus/median eminence area and subsequently release the peptide into portal blood bathing the adenohypophysis, but they also project to the MCG, the site mediating sexual receptivity. GnRH applied to the MCG stimulates sexual receptivity, whereas GnRH antisera applied to this area depresses sexual receptivity.

Aside from neuropeptides, pituitary hormones themselves may play a role in sexual receptivity. Indeed, prolactin applied directly to the MCG enhances sexual receptivity in rats receiving a low dose of estrogen. Conversely, pharmacologic depression of prolactin secretion at the time of anticipated onset of estrus depresses the magnitude of sexual receptivity. Finally, pituitary hormones are released in response to the mating stimulus. It is well established that prolactin is released from the adenohypophysis of rodents in response to excitation at the uterine cervix by the act of mating, which is transmitted to the hypothalamus via spinal pathways. It has been shown that the mating stimulus acts at the hypothalamus by lowering tuberoinfundibular dopaminergic tone, which subsequently leads to the release of prolactin. Prolactin, in turn, activates the corpora lutea to maintain progesterone secretion, which maintains the subsequent pregnancy.

The other pituitary hormone released in response to the mating stimulus is oxytocin. Once again the

Table 24
Hypothalamic Control of Sexual Behavior in Males

<i>Chemical mediator</i>	<i>Site of action</i>	<i>Effect</i>
Testosterone	Preoptic area	Control consummatory behaviors
Estradiol	Amygdala	Control motivational behaviors
Mesolimbic dopamine	Amygdala	Control motivational behaviors
Incertohypothalamic	Preoptic area	Control consummatory behaviors
GnRH	Preoptic area	Control consummatory behaviors
Endorphins	Preoptic area	Inhibit consummatory behaviors

stimulus is transmitted over spinal pathways to enhance the activity of magnocellular neurons in the paraventricular nucleus. The mating-induced release of oxytocin in females (and vasopressin in males) contributes to affiliative social behavior in some species. In meadow voles, for example, life-long pair bonding between the female and male is mediated by OT/VP release.

4.3.3.2. Hypothalamic Control of Sexual Behavior in Males. The sexual behavior of male rodents is characterized as having both *motivational* and *consummatory* components (Table 24). Motivational are those behaviors necessary to gain access to the female in heat. Consummatory are those behaviors necessary for copulation. These would include mounting, erection, intromission, and ejaculation. Stereotypical male sexual behaviors are provoked by testosterone secreted from the Leydig cells of the testis. Testosterone controls male sexual behavior through two mechanisms. In peripheral tissues, testosterone is converted to dihydrotestosterone (DHT). DHT is responsible for stimulating sensory receptors and thus may play a role in penile erection. Testosterone acts on the preoptic area (POA) of the hypothalamus to integrate the various consummatory components of male sexual behavior. The amygdala controls the motivational components of male sexual behavior. This appears to be a function of estrogen that has been aromatized from testosterone intraneuronally. Among the catecholamines, dopamine from mesolimbic neurons appears to be the neurotransmitter controlling the motivational component of male sexual behavior. The incertohypothalamic dopaminergic system appears to be responsible for the consummatory component. Neuropeptides have been shown to modulate both motivational and consummatory behaviors. GnRH appears to act within the POA to control consummatory behaviors. Endorphin neurons projecting from the amygdala to the POA appear to have the opposite effect of inhibiting many consummatory behaviors. Other peptides that

have been implicated in male sexual behaviors include substance P, NPY, α MSH, and oxytocin. However, the physiologic significance of their role has not been fully determined.

As mentioned earlier, the POA of the hypothalamus is sexually dimorphic, which is reflected in the pattern of LH secretion from the adenohypophysis. In addition, the dimorphic nature of the hypothalamus is also reflected in stereotypical male or female sexual behaviors. Just as males who are deprived of testosterone neonatally present a female cyclic pattern of LH secretion when challenged with estrogen as adult, so they will also present the typical female receptive lordotic pattern in response to estrogen and an aggressive male. Conversely, a female treated neonatally with testosterone will show the noncyclic pattern of LH secretion when adult and, if treated with testosterone as adult, will mount females in heat. Some of this sexual differentiation occurs *in utero* but in rodents most of it is determined neonatally. Thus the hypothalamus develops potentially as functionally female while the differentiating event is the presence of androgen prenatally or neonatally.

4.3.4. MATERNAL BEHAVIOR

The hypothalamus is also intimately involved in mediating maternal behaviors stimulated by both ovarian and pituitary hormones (Table 25). Once again, the rodent model is the most frequently studied. There are essentially four components of maternal behavior in the rat. *Nest building* is the first behavioral sign. Late in pregnancy, the rat will gather bedding and any other materials available to it and prepare a nest in which she can deliver, nurse, and care for her pups. She designs this to be the center of all her activities while the pups are present. After the pups are born, the dam spends a large amount of time *licking* the neonates for the purpose of cleaning. The typical behavior rodents share with all mammals is assumption of a *nursing* posture to allow the hungry pups access to the mammary glands for retrieval of milk. Finally, as the nursing pups mature, they tend to leave the convenience and safety of the mother's

Table 25
Hypothalamic Control of Maternal Behavior

<i>Chemical mediator</i>	<i>Site of action</i>	<i>Effect</i>
Estrogen after decline of progesterone	Medial preoptic area	Stimulate maternal behaviors
Prolactin	Preoptic area	Stimulate maternal behaviors in estrogen-primed rats
Oxytocin	Ventromedial nucleus	Stimulate maternal behaviors in estrogen primed rats

nest. The nursing mother then spends much time retrieving the pups to the nest.

The hormonal drive for the onset of maternal behavior actually occurs during the prepartum period (Table 25). By supplying foster pups late in pregnancy, the development of these behaviors can be characterized. The signal appears to be the gradual decline in progesterone secretion from the corpus luteum (or from the placenta in non-rodent species) coupled with the increase in ovarian estrogen secretion as the time of parturition approaches. The prepartum period can therefore be envisioned as a period of *hormonal priming*. Maternal behaviors are not only due to the combined actions of estrogen in the face of the withdrawal of progesterone, but they are also influenced by adenohypophyseal (or perhaps even neural) prolactin. In addition, oxytocin may play a role as well.

Estrogen exerts its effect on maternal behavior largely through an action at the medial preoptic area (MPOA) of the hypothalamus. Much of the action of estrogen at the MPOA is through stimulation of estrogen receptors. In general, throughout pregnancy estrogen receptors are much greater in the POA than in the entire hypothalamus. On the last day of pregnancy, estrogen receptors in the rest of the hypothalamus rise to levels equivalent to those of the POA.

In addition to stimulating parental behaviors directly, estrogens also stimulate prolactin secretion. Moreover, it is the withdrawal of progesterone at the end of pregnancy that allows prolactin to exert its actions on the mammary gland to initiate and maintain lactation. Prolactin, secreted after parturition, has been implicated in the control of maternal behaviors. Indeed, hypophysectomy or treatment with the dopamine agonist bromocryptine will prevent many of the components of maternal behaviors. Similarly, mice lacking prolactin receptors show markedly impaired maternal behavior. In contrast, infusion of prolactin directly into the POA or into the CSF through the third ventricle stimulates maternal behaviors in estrogen-primed female rats. Thus prolactin can act upon cells in the POA as well as the circumventricular organs. Because prolactin is a large polypeptide, it is unlikely that it can cross the blood-brain barrier to affect neural structures. There are essentially three possibilities from the route prolactin may take to affect neural structures and subsequently maternal behavior. One is that pituitary prolactin arrives at the hypothalamus by *retrograde blood flow* through the portal circulation. Alternatively, it has been shown that circulating prolactin has access to

the CSF and brain through a receptor-mediated transport system located in the choroid plexes of the lateral, third, and fourth ventricles. Finally, the most recent evidence is that specific areas of the hypothalamus contain prolactin mRNA leading to the suggestion that prolactin is synthesized in these areas distinct from pituitary prolactin. Because estrogen can enhance brain and CSF levels of prolactin in *hypophysectomized rats*, it has been suggested that estrogen stimulates central prolactin synthesis and that centrally prolactin may enhance the sensitivity of estrogen-sensitive cells in the hypothalamus that regulate maternal behavior.

Among the hypothalamic peptides, oxytocin (OT) has been shown to promote maternal behavior when injected into the CSF of estrogen-treated rats. OT is ineffective when injected peripherally. Moreover, an OT antagonist is effective in delaying maternal behavior when injected centrally. Destruction of the paraventricular nucleus, the source of OT, also modifies maternal behaviors. The effects of OT are correlated with the appearance of OT cell membrane receptors in areas of the brain known to mediate maternal behavior. These include the VMN of the hypothalamus, the bed nucleus of the stria terminalis, the anterior olfactory nucleus, and the central nucleus of the amygdala.

4.3.5. EMOTIONAL BEHAVIORS

It has long been appreciated that the hypothalamus participates in emotional responses. For example, electrical stimulation of the lateral hypothalamus of cats results in many of the somatic and autonomic characteristics of *anger* such as piloerection, pupillary constriction, arching of the back, raising of the tail, and increased blood pressure. Similar rage-like responses can be elicited by decortication or merely separating the hypothalamus from the cortex. The anger that is elicited is referred to as *sham rage*. Such animals respond to seemingly innocuous stimulation with a multitude of aggressive responses. The hypothalamus appears to act as an integrating center for these responses.

4.4. Regulation of Rhythmic Events

4.4.1. TYPES OF RHYTHMS

Rhythms (Fig. 29) are characterized by their *period* (the time to complete one cycle), *frequency* (number of cycles per unit time), *phase* (points of reference on a timescale), and *amplitude* (the magnitude of variation from the mean). Rhythms are synchronized or *entrained* with the external environment by *zeitgebers*

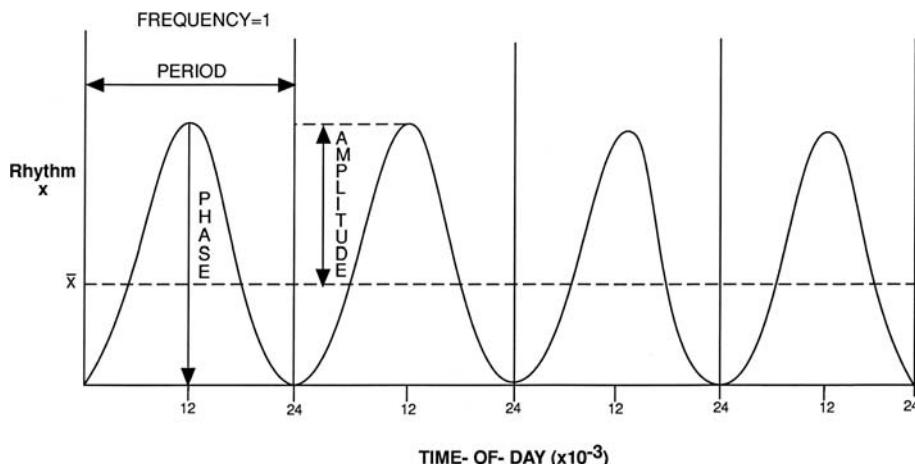


Fig. 29. Parameters of a *rhythm* (x) over 4 complete cycles of any *zeitgeber*. For purposes of this example, imagine that the *zeitgeber* is the lighting periodicity of an artificial 24-h day with daylight lasting from 7 A.M. to 7 P.M. The *period* is the time to complete one cycle of the rhythm. In this example, the period is 24-h. The *frequency* is the number of cycles per unit of time. Here the frequency of rhythm x is 1 (per day). The *phase* is the maximum of the rhythm in reference to a timescale such as that provided by the lighting periodicity or a clock. The *amplitude* is the deviation from the mean of the rhythm, \bar{x} .

Table 26
Categories of Biological Rhythms

Rhythm	Approximate period	Example
Ultradian	Much less than 24 h	Respiration, heart rate
Circadian	Approximately 24 h	Corticosterone rhythm
Infradian	Greater than 24 h but much less than 365 days	Menstrual cycles
Circannual	Seasonal, approximately 365 days	Hibernation

(“time-givers”) such as the light/dark periodicity provided by the earth’s rotation. Many biological rhythms are endogenous and self-sustaining and thus persist even when animals are maintained under constant conditions (e.g., constant dark).

Biological rhythms fall into one of four categories based on their period (Table 26): *circadian* (approximately a day and usually synchronized with the day-night lighting cycle), *ultradian* (less than a day and of much greater frequency such as heartbeat or respiration), *circannual* (greater than a day and usually synchronized with seasonal events such as seasonal fat deposition), and *infradian* (greater than a day but shorter than a year such as menstrual or estrous cycles).

4.4.2. ROLE OF THE HYPOTHALAMUS IN BIOLOGICAL RHYTHMS

We have already described the role of hypothalamic neurohormones and neurotransmitters in

infradian rhythms characterized by the menstrual and estrous cycles. Indeed, the cyclic release of luteinizing hormone every 28 days in the human female involves participation of parts of the hypothalamus ranging from the most rostral to the most caudal boundaries.

4.4.2.1. Suprachiasmatic Nucleus Is the Central Circadian Clock. Hypothalamic circadian rhythms require the participation of a timing device or *clock* within the hypothalamus that coordinates diverse rhythms and synchronizes them with the external environment. This role is served by the *suprachiasmatic nucleus* (SCN) of the hypothalamus. The SCN has diverse projections that provide timing information to rhythms of sleep-wake, body temperature, hormone release, and so forth. Destruction of the SCN will result in the inability of the rat to generate coherent circadian rhythms (although ultradian

rhythms will continue to be expressed). The SCN contains a self-sustaining clock, because SCN explants will continue to fire *in vitro* in a rhythmic pattern with an approximately 24-h period. The genomic basis for the SCN's rhythmicity has been established, derived largely from the discovery of mutant mice missing specific genes and expressing aberrant circadian rhythms. A transcriptional feedback network within SCN neurons involves the expression of activator proteins such as CLOCK and BMAL (“brain and muscle aryl hydrocarbon receptor nuclear translocator-like”) that enhance neurotransmitter synthesis and neuronal metabolism. At the same time, CLOCK and BMAL also stimulate the expression of repressor proteins such as PER (“period”) and CRY (“cryptochrome”). Over the course of the circadian cycle, PER and CRY levels rise until they turn off the expression of CLOCK and BMAL and thus suppress neuronal activity. Once CLOCK and BMAL expression is suppressed, then PER and CRY expression also falls, allowing CLOCK and BMAL levels to rise, and the cycle repeats.

A circadian rhythm generalizable to virtually all mammals is the adrenal corticosterone rhythm. In response to entrainment by lighting periodicity (rodents) or activity rhythms (man), corticosterone levels in the blood begin to increase and reach peak magnitudes at the same time each day. This has been shown to be driven by pituitary ACTH and hypothalamic CRH as described previously. In rodents, the peak occurs at the onset of darkness, whereas in man it is the onset of activity or wakefulness cycles. Clinically, an awareness of circadian fluctuations in hormone levels is critical to ensure accurate measurement.

4.4.2.2. Sleep and Arousal. The most obvious behavioral circadian rhythm is that of sleep and wakefulness. Sleep and wakefulness is regulated by a hypothalamic-midbrain-pontine network. The ascending arousal system includes projections to the hypothalamus, thalamus, and cortex from noradrenergic cells of the LC, serotonergic cells of the dorsal and median raphe (DR and MR), and histaminergic cells of the tuberomammillary nucleus (TMN). These projections are active during wakefulness and rapid-eye movement (REM) sleep. In particular, the ascending arousal system inhibits the activity of neurons in the ventrolateral preoptic nucleus (VLPO). In contrast, VLPO neurons are active during sleep and have inhibitory projections back to the components of the ascending arousal system. In addition, a population of neurons in the lateral hypothalamus that express orexin/hypocretin project

widely throughout the brain and are prominently connected with the LC, TMN, and raphe nuclei. Although it has several behavioral effects, orexin is a major stimulator of arousal. Mice, dogs, and the few humans with mutations in the orexin type 2 receptor are narcoleptic, meaning that they spontaneously pass from wakefulness into REM sleep. Furthermore, most human narcoleptics have a very discrete loss of orexin neurons, perhaps secondary to autoimmune-mediated neurodegeneration.

4.4.3. PULSATILE ULTRADIAN RHYTHMS

It is quite clear that the secretion of most pituitary hormones is not just biphasic (basal and surge pattern) but is actually pulsatile, and blood levels of hormone at any time represent the summation of an ultradian pattern of hormone secretion from the cell. Such a rhythm is probably the consequence of the activity of a *pulse generator* within the hypothalamus regulating neurohormone secretion into hypophyseal portal blood. A pulsatile ultradian rhythm of LH secretion is revealed in ovariectomized rats and monkeys. Measurement of multiple unit activity in the medial basal hypothalamus of monkeys reveals that the pulsatile pattern of LH secretion coincides with spikes of multiple unit activity in the medial basal hypothalamus. This implies that the pulse generator for GnRH and subsequent LH secretion (at least in monkeys) resides within the medial basal hypothalamus.

4.4.4. CIRCANNUAL RHYTHMS

Annual or circannual rhythms are not exclusively linked to the hypothalamus. Annual behavioral rhythms are of two types. Type I annual rhythms are dependent upon the environment and type II are dependent upon an endogenous biological clock. Type I rhythms are generally photoperiodic driven in that they require transduction of seasonal changes in day length. For example, as day length shortens during the late summer through early fall (short days), voles reduce their food intake and their gonads involute. Under long days of spring, food intake and gonadal weights return to normal. Type II annual rhythms are those that *free run*, that is, require no environmental input and thus persist under constant environmental conditions. European starlings store fat prior to their demanding spring migration. Under constant light, temperature and food availability, the rhythm of fat deposition persists. Each animal free runs with a period of 1 year and eventually becomes

desynchronized under constant environmental conditions.

We have already mentioned that photoperiodic time measurement involves the SCN of the hypothalamus. Fibers from the retina of the eye terminate within the SCN. It is over this *retinohypothalamic tract* that the lighting periodicity is transduced. Efferent fibers from the SCN terminate in the paraventricular nucleus, which in turn sends efferent fibers via the medial forebrain bundle to the spinal cord that terminate upon the *intermediolateral cell column*. Processes from these cells synapse in the *superior cervical ganglion of the sympathetic chain*. Postganglionic noradrenergic fibers from this area then project to and innervate the *pineal gland*. By this pathway, the SCN generates a circadian rhythm in the pineal hormone *melatonin* that is synchronized to the light-dark cycle. Melatonin appears to be most important in mediating effects of annual rhythms. Because melatonin is produced in greatest amounts during dark phases of the cycle, melatonin level is inversely correlated with day length and thus transduces seasonal information. The testes of male hamsters are most competent to produce sperm and normal levels of testosterone during the long days of summer. Pinealectomy prevents the loss of competency when animals are placed in the abbreviated illumination of short days. However, if pinealectomized hamsters receive long melatonin pulses (signaling short days or long nights), the testes regress independent of the environmental photoperiod. There appears to be a strain difference in the sensitivity to melatonin. In addition, not all mammals are dependent upon the pineal gland for generation of biological rhythms. For example, the rat, a photoperiodic mammal, has normally timed ovulation-inducing surges of LH release from the pituitary gland when pinealectomized. In those animals responsive to melatonin, the sites in the central nervous system and periphery where melatonin acts to regulate biological rhythms is unclear. Though there are marked species differences, the anterior hypothalamus, the SCN, and the adenohypophysis seem to be candidate sites.

It is particularly useful to appreciate the multifaceted roles of various areas of the hypothalamus

regulating the various rhythms. For example, control of reproduction in the rodent is subject to numerous different rhythms. It is well-known that basal GnRH secretion shows ultradian variation, being pulsatile in nature with a periodicity of 30 min to 4 h, depending on levels of steroid negative feedback. In addition, GnRH neurons in the POA respond to rising levels of estrogen secreted every 4 to 5 days during the estrous cycle by secreting the peptide into portal blood and consequently release a large bolus of LH into peripheral plasma to induce ovulation. Thus the POA controls an infradian rhythm. The POA also controls a circadian rhythm. Again, in rodents, ovariectomy and estrogen replacement results in not just a single preovulatory-like *surge* of LH secretion, but a surge at the same time on each day for the next several days. Shifting the lighting phase will shift the time of the occurrence of each surge an equivalent amount. Thus, the lighting periodicity is the *zeitgeber* and the biological event is a true circadian rhythm. Finally, in some species, reproduction is seasonally regulated, meaning that the whole GnRH/LH/gonadal system can be subjected to a circannual rhythm.

5. CONCLUSION

By now, the neuroscience student must appreciate a point made in Section 1 of this chapter. The hypothalamus does not exert control over one process exclusive of other processes. That is, the hypothalamus plays an *integrative role* in adapting the organism to demands put upon it by its environment. Because of its multifaceted role in allowing the organism to respond to the environment, the hypothalamus can be appropriately characterized as an organ that most uniquely ensures the perpetuation of the species.

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Michael W. Miller and Brent A. Vogt

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1. INTRODUCTION

As the name implies, the cerebral cortex forms a shell that covers the brain. In fact, cortex forms most of the visible surface of the brain. Below its surface is a complex network of neurons, their axons, and glia. The cerebral cortex is not uniform; rather, it is composed of many structurally and functionally unique subunits that perform a wide range of sensory, motor, and mnemonic processes associated with cognition. The cerebral cortex organizes affective behaviors including responses to painful stimuli, maternal and sexual behaviors, and the expression of rage and other emotions. Like other parts of the CNS, the cerebral cortex does not function in isolation; it is a part of an intricate plexus of overlapping circuits. In this chapter, you will be acquainted with the morphology of cortical neurons and the way in which these neurons are assembled into clusters, columns, and areas. In addition, this chapter describes the

intrinsic and extrinsic circuitries that subserve the principal functions of the cerebral cortex.

2. SURFACE FEATURES OF THE CEREBRAL CORTEX

Composite magnetic resonance images of the cerebral cortex, like those in Fig. 1, show that the cortex has a corrugated appearance. The crest of each fold is termed a gyrus, and the depression, or cleft, between adjacent gyri is a sulcus. Very deep sulci are also termed fissures, and these include the space between the hemispheres or interhemispheric fissure and the lateral fissure (of Sylvius). The lateral surface of the cerebral cortex is composed of four lobes, the frontal, parietal, temporal, and occipital lobes. The medial surface contains extensions of each of these lobes and the limbic lobe.

The *frontal lobe* is the largest segment of the cerebral cortex. It extends from the rostral pole of each hemisphere to the central sulcus (of Rolando) and from the cingulate sulcus on the medial wall to the lateral fissure on the lateral surface. The frontal lobe

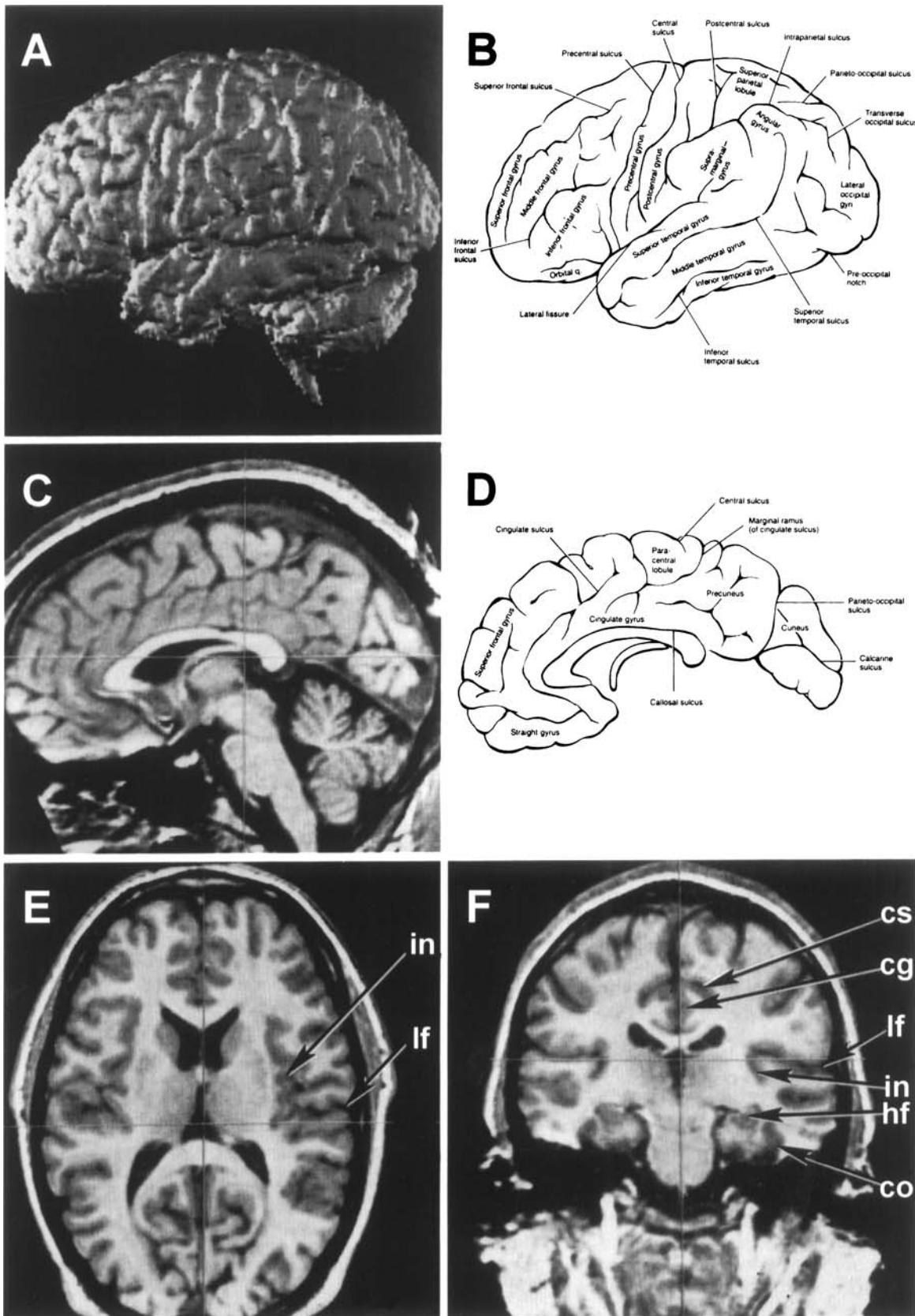


Fig. 1. (Continued)

is composed of five major gyri. (1) The precentral gyrus, which contains motor cortex, runs parallel to the central sulcus and is bounded by the central and precentral sulci. (2–4) Coursing perpendicular to the precentral gyrus on the lateral surface of the frontal lobe are the superior, middle, and inferior frontal gyri. The inferior frontal gyrus is further subdivided into the opercular, triangular, and orbital parts. An operculum is an extension of cortex that overlies or overhangs another region. In this instance, the operculum overlies cortex in the depths of the lateral fissure, the insular cortex (Fig. 1E, F). The opercular and triangular divisions of the inferior frontal gyrus contain Broca's speech area. (5) The fronto-orbital gyri form the ventral part of the frontal lobe and rest on the orbital plate of the frontal bone.

The *parietal lobe* is bounded rostrally by the central sulcus, medially by the cingulate sulcus, posteromedially by the parieto-occipital sulcus, posterolaterally by an imaginary line between the parieto-occipital sulcus and the preoccipital notch, and ventrally by the lateral fissure. Parietal cortex includes the postcentral gyrus, a strip of cortex that is located posterior to the central sulcus and parallel to the precentral gyrus. The postcentral gyrus contains somatosensory cortex. Caudal to the postcentral gyrus are the superior and inferior parietal lobules that are divided by the intraparietal sulcus. The inferior parietal lobule is composed of two gyri, the supramarginal gyrus, which caps the lateral fissure, and the angular gyrus, which straddles the border with occipital cortex and forms the banks of the end of the superior temporal sulcus. Two regions form the medial surface of the parietal lobe. One is the paracentral lobule, which surrounds the medial tip of the central sulcus. This lobule also includes a segment of frontal cortex. Caudal to the paracentral lobule is the precuneal cortex, which ventrally blends with the limbic lobe.

The *insula* is an island of cortex in the depths of the lateral fissure. It is bounded by cortex of the frontal, parietal, and temporal lobes. The dorsal border of the lateral fissure is composed of the opercular

part of the inferior frontal gyrus and the parietal operculum, whereas the ventral border is composed of the temporal operculum. The rostral insula is part of the limbic system, although its specific functions are not fully understood. The posterior part of the insula is involved mainly in processing somatosensory information.

The *temporal lobe* is ventral to the lateral fissure. This lobe includes the superior, middle, and inferior temporal gyri that run parallel to the lateral fissure. The dorsal plane of the superior temporal gyrus contains the transverse gyri (of Heschl) where the primary representation of audition is located. The ventral part of the temporal lobe includes the occipito-temporal gyrus. The *occipital lobe* includes cortex caudal to an imaginary line through the parieto-occipital sulcus and notch, which demarcates it from the temporal lobe. The occipital lobe includes the calcarine fissure within and around which is visual cortex.

In 1868, the comparative neurologist Paul Broca defined the *limbic lobe*. This lobe is composed of the cingulate gyrus and hippocampal and parahippocampal gyri. The limbic lobe contains areas that are a major part of the *anatomic limbic system* as discussed in Chapter 16. This cortical region is involved in olfaction, memory, and visceral, skeletal, and endocrine functions associated with emotional behaviors.

3. CORTICAL CYTOLOGY

3.1. *Projection Neurons*

Most cortical neurons are projection neurons, and the most common cortical projection neuron is the pyramidal neuron. The general features of projection neurons are described in Table 1. A typical pyramidal neuron has a large pyramid-shaped cell body that gives rise to two sets of dendrites (Fig. 2). One set is a prominent dendrite that issues from the apex of the cell body. The apical dendrite often reaches layer I where it arborizes in a tuft of dendrites. In addition, the apical dendrite gives rise to smaller-caliber

Fig. 1. (Continued)

Magnetic resonance imaging is used to examine the structure of the brain *in vivo*. (A) A lateral view of the cerebral cortex can be appreciated in a brain reconstructed from serial magnetic resonance images. This rendered image is a compilation of a series of 1.5-mm optical sections. (B) The sulci and gyri, which can be identified on the lateral surface, are labeled. The labels for the sulci are placed around the brain, whereas the labels for the gyri are placed at the appropriate site on the brain. (C, D) A parasagittal section reveals a number of the features characteristic of the medial surface of the brain. The horizontal and vertical lines shown in (C) identify the planes of section used in the horizontal and coronal sections shown in (E) and (F), respectively. cg, cingulate gyrus; co, collateral sulcus; cs, cingulate sulcus; hf, hippocampal formation; in, insula; lf, lateral fissure. (Magnetic resonance images courtesy of Nancy Andreasen, University of Iowa.)

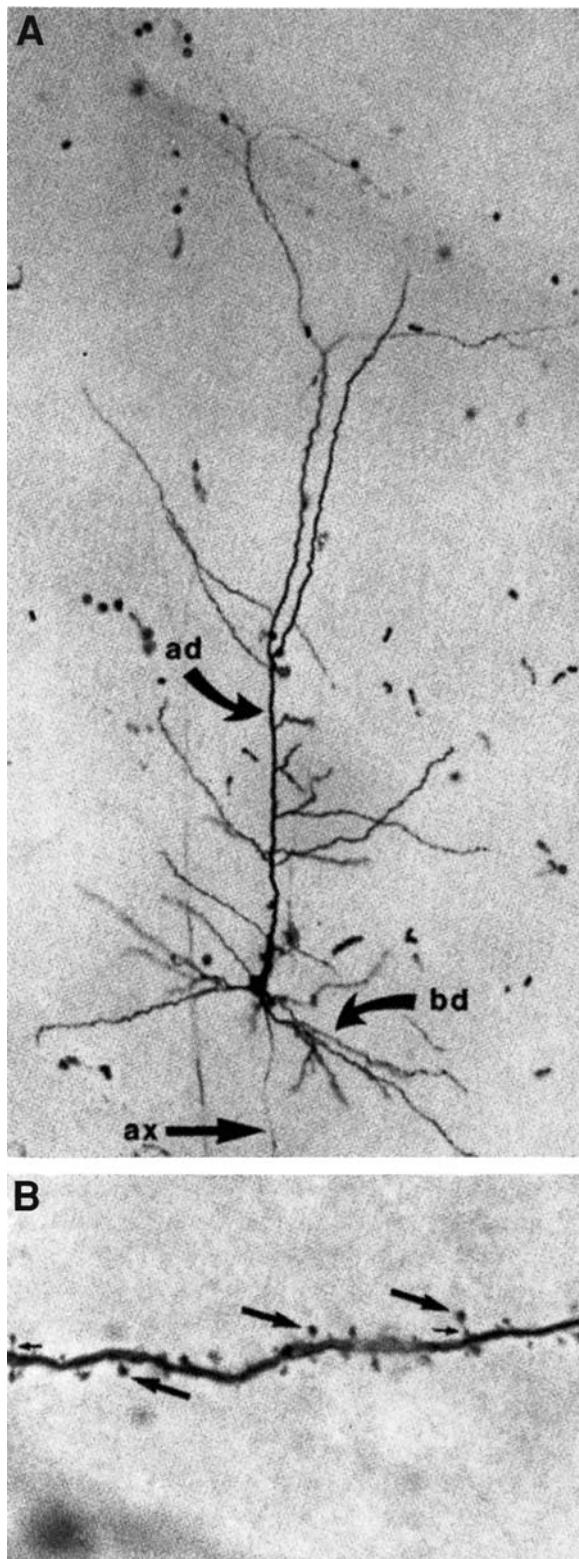


Fig. 2. (A) This pyramidal neuron was intracellularly injected with the tracer, horseradish peroxidase, which was subsequently localized histochemically. This neuron has an apical dendrite (ad) that ascends from the apex of the pyramid-shaped cell body and reaches layer III. At this point, it branches to form an apical tuft that ramifies within layers I and II. The base of the cell body gives rise to a set of dendrites (bd) and an axon (ax) that descends toward the white matter. (B) Each dendrite is invested with a coat of spines. A spine has a bulbous head (*large arrows*) that is attached to the dendritic shaft by a long, thin neck (*small arrows*).

Table 1
Features of Types of Cortical Neurons

Feature	Projection neurons	Local circuit neurons
Dendrites	Spinous	Aspinous
Axons	Local arbors and distant projections	Local arbors only
Synapses formed by:		
Axo-somatic afferents	Symmetric	Symmetric and asymmetric
Efferents	Asymmetric	Symmetric
Neurotransmitter	Glutamate and aspartate	GABA and neuropeptides
Discharge properties	Regular spiking	Fast spiking

collateral processes that often branch at 90° angles. The second set of dendrites is processes that emanate from the base of the cell body. In contrast with the apical dendrite, the branches of the basal dendrites bifurcate at acute angles. All of the dendrites (with the exception of the dendritic segments proximal to the cell body) are densely covered with small protuberances known as spines. A spine typically has the appearance of a lollipop with a large rounded head that is attached to the dendritic shaft by a slender neck.

Apical dendrites of pyramidal neurons are often aggregated together into clusters. Each cluster contains the apical dendrites of pyramidal neurons whose cell bodies are distributed in superficial and deep cortex. The apical dendrites of neurons whose cell bodies are in deep cortex form the core of the cluster, whereas the apical dendrites of neurons whose cell bodies are in superficial cortex are distributed at the periphery of the cluster. Such clusters may define a functional unit or module.

With few exceptions, the axons of pyramidal neurons arise from the base of the cell body. In addition to emitting a local plexus of collaterals, the axons of pyramidal neurons have one of two projection patterns. They either (1) project from one region of cortex to another in the ipsilateral cortex (association projections) or to contralateral hemisphere (callosal projections) or (2) descend to subcortical structures. The axons of projection neurons release the excitatory amino acids aspartate and/or glutamate as a neurotransmitter.

There are two subpopulations of projection neurons that do not have apical dendrites. Notable among these are the spinous stellate neurons in the intermediate zones of sensory cortices and the large, nonoriented neurons in superficial entorhinal cortex. Like the typical pyramidal neurons, these projection neurons have spinous dendrites, and they excite the postsynaptic neurons through the release of glutamate.

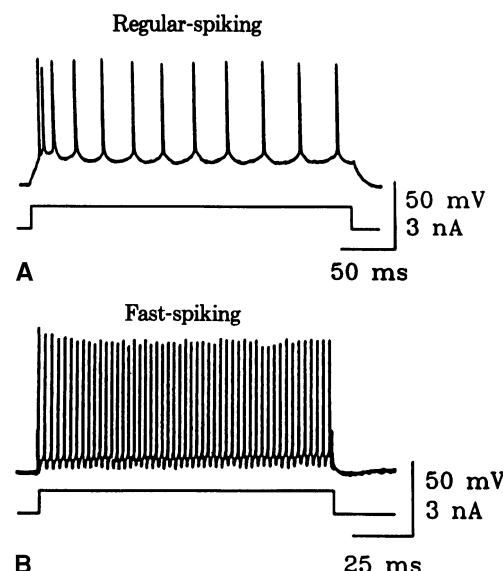


Fig. 3. Intracellular recordings show that projection and local circuit neurons exhibit different firing patterns. (A) Projection neurons have a phasic, regular spiking pattern. (B) Stimulation of a local circuit neuron results in a continuous and maintained stream of fast spikes. (Reproduced with permission, Connors and Gutnick, Trends Neurosci. 1990;13:99–104.)

Most projection neurons respond to a stimulus with a series of spikes that are followed by prolonged after-hyperpolarizations and after-depolarizations (Fig. 3). These regular spiking neurons adapt to sustained stimuli.

3.2. Local Circuit Neurons

Based on their somatodendritic morphology, two groups of local circuit neurons can be discerned. One group is the stellate neurons. The stellate neurons have round cell bodies and an array of dendrites that radiate uniformly from their somata (Fig. 4). The second group of local circuit neurons has a polarized form. Their cell bodies are elongate and may be oriented either radially or horizontally. Regardless of

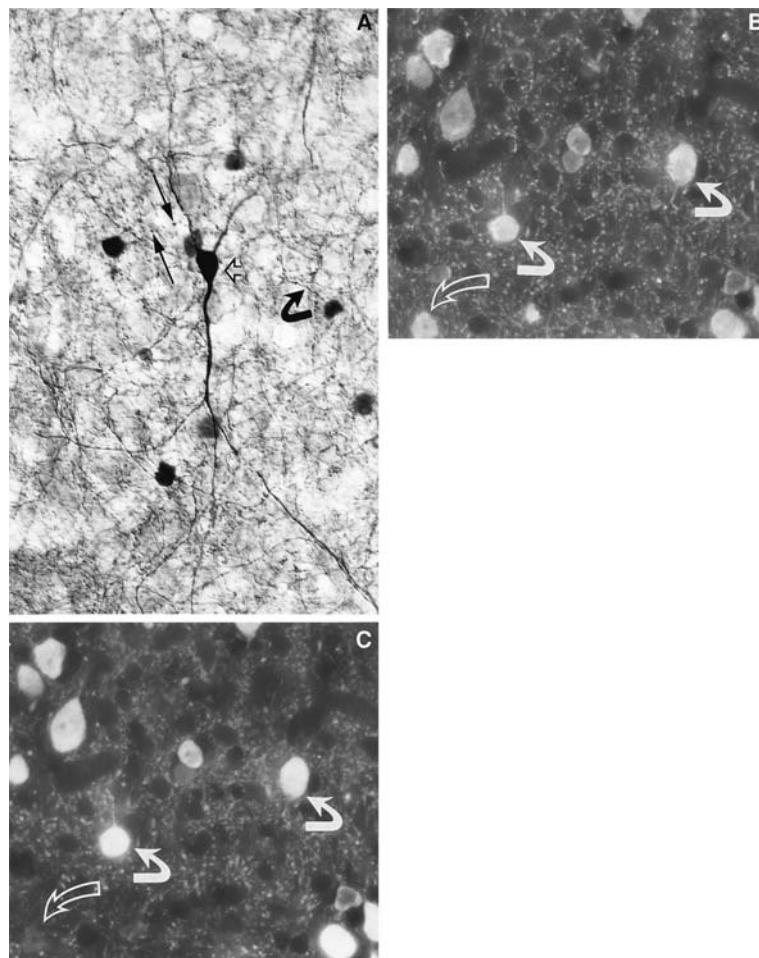


Fig. 4. (A) Cortical local circuit neurons use γ -aminobutyric acid (GABA) as a neurotransmitter. The *open arrow* indicates the cell body of an aspinous stellate neuron that was labeled immunohistochemically with an antibody directed against glutamic acid decarboxylase (GAD). GAD is the enzyme that catalyzes the rate-limiting step in GABA synthesis. GAD-immunoreactivity is evident in the axonal processes (*curved solid arrow*) and in GABAergic axonal terminals (*straight solid arrows*). Many GABAergic neurons colocalize with a peptide neurotransmitter. Immunofluorescence techniques were used to identify GABA-immunoreactive neurons (indicated by *curved solid arrow* in B), which were double-labeled with an anti-substance P antibody (indicated by corresponding *curved solid arrows* in C). Note that not all of the GABAergic neurons colocalize substance P (*open arrows* in B and C). (Courtesy of Stewart Hendry, Johns Hopkins University.)

their orientation, the dendrites tend to arise from the attenuated poles of the cell body. The dendrites of all local circuit neurons are aspinous or at most sparsely spinous.

The pattern of the axonal arbors of local circuit neurons is quite variable. The axons may arise from virtually any site on the cell body or from a proximal dendrite. The distribution of these axons is restricted to the sphere of the dendritic arbors or extends beyond the dendritic field. Apparently all local circuit neurons use γ -aminobutyric acid (GABA) as their neurotransmitter (Fig. 4A). Release of this neurotransmitter inhibits the activity of postsynaptic neurons. Local circuit neurons can release other neuroactive substances from their axonal terminals

(Fig. 4, C). These peptides include acetylcholine, cholecystokinin, neuropeptide Y, somatostatin, substance P, and vasoactive intestinal polypeptide. It is not yet clear how these other neuroactive substances interact with GABA on postsynaptic neurons to modulate neurotransmission. On the other hand, it does appear that the release of the secondary substances is activity dependent. That is, at low levels of excitation, local circuit neurons release only GABA, whereas at high levels of excitation, they release both GABA and the other neuroactive compound.

Intracellular recordings of local circuit neurons reveal that cortical local circuit neurons have membrane and spiking properties that differentiate them from projections neurons. After a suprathreshold

stimulation, local circuit neurons discharge fast spikes of less than 0.5 ms duration (Fig. 3). These neurons exhibit little or no adaptation during a prolonged stimulation (i.e., the spike frequency remains the same). Thus, the local circuit neurons transmit inhibitory information to postsynaptic targets with great fidelity.

3.3. Cortical Synaptology

Two structurally and functionally unique types of synapses are formed by cortical neurons. *Asymmetric synapses* are formed by axons that contain large vesicles and excitatory neurotransmitters (i.e., primarily glutamate). These synapses have postsynaptic densities that are composed of a protein kinase, and activation of these synapses leads to depolarizing potentials or excitatory responses in postsynaptic neurons. In contrast, *symmetric synapses* have presynaptic axons with small synaptic vesicles that contain the inhibitory transmitter GABA. The presynaptic and postsynaptic densities of these synapses are approximately of equal thickness. Activation of symmetric synapses evokes hyperpolarizing potentials or inhibitory responses in postsynaptic neurons.

The distributions of asymmetric and symmetric synapses are among the many features that distinguish projection from local circuit neurons. The two types of synapses are largely segregated by projection neurons. The most common target of axons that form asymmetric synapses is the heads of dendritic spines. In contrast, most symmetric synapses are formed in the perisomatic region. This region includes the soma, the smooth surfaces of proximal dendrites, the axon hillock, and the initial segment of the axon. The result of this organization is that excitatory responses can be evoked over a large area of the pyramidal cell dendritic tree. The summed excitatory input is gated by inhibitory activity in the perisomatic region. Because the zone for the initiation of an action potential is in the initial segment of the axon and the axon hillock region of the cell body, the symmetric synapses are strategically placed to modulate the discharge frequency of cortical pyramidal neurons. In contrast, asymmetric and symmetric synapses are intermingled along the smooth dendrites of local circuit neurons. The dispersion of inhibitory synapses in relationship to excitatory ones results in a less pronounced gating of excitatory activity by local circuit neurons.

The efferents of the two classes of cortical neurons have different patterns of connectivity. The local arbors and projections of pyramidal neurons form excitatory, asymmetric synapses with postsynaptic targets. The axons of local circuit neurons form

inhibitory, symmetric synapses. Thus, the two neuronal populations affect their targets in opposite ways.

The functional and connectional differences of the two populations of cortical neurons are evident during an epileptic seizure. In the absence of local circuit neuron-mediated inhibition, the activity of the projection neurons is unchecked. Projection neurons discharge without inhibitory modulation and produce depolarizing shifts that are composed of very large excitatory postsynaptic potentials. A common treatment for seizure activity is administration of compounds that have actions similar to GABA such as valproic acid.

4. STRUCTURE AND DISTRIBUTION OF CORTICAL AREAS

The size and packing densities of neurons are not uniform in the cerebral cortex. *Cytoarchitecture* refers to the unique distributions of neurons in different parts of the cortex. The underlying tenet of this appreciation of cortical organization is that structural differences in the cerebral cortex are associated with functional unique areas. Figure 5 has Nissl-stained sections through somatosensory and motor

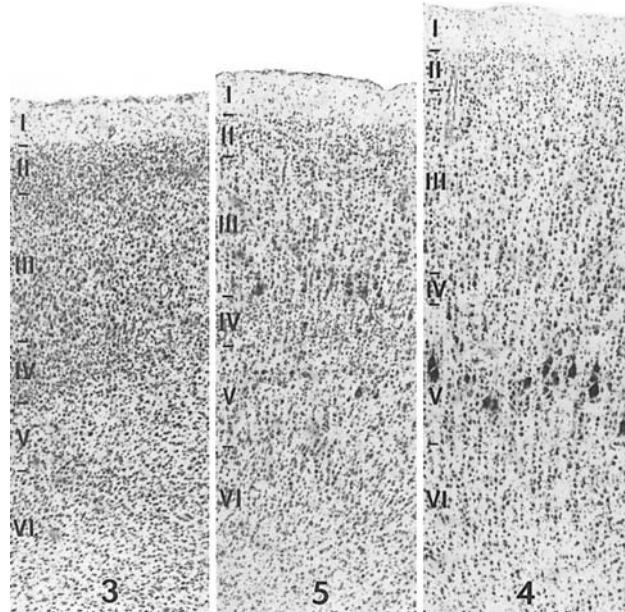


Fig. 5. Photomicrographs of Nissl-stained sections through three sensorimotor areas to show differences in cytoarchitectural organization: primary somatosensory cortex, area 3; first somatosensory association cortex, area 5; motor cortex, area 4. Notice the highly granular layers II and IV in area 3 and large pyramidal neurons in the deep part of layer III in area 5 and in layer V of area 4.

cortices (i.e., areas caudal and rostral to the central sulcus, respectively). One of the striking features of cortical architecture is the horizontal alignment of its neurons into layers. It is generally accepted that neocortex or *isocortex* contains six layers.

The differentiation of cortical layers is largely based on the distinctive population of projection neurons in a layer. (Note: A neuron is described as being in a layer based on the position of its cell body. Thus, a pyramidal neuron with a cell body in layer V is referred to as a layer V neuron, or a layer may be described as having pyramidal neurons.) The most superficial cortical layer (i.e., the layer that abuts the pia mater) is layer I. Layer I is also referred to as the plexiform or molecular layer. Layer I is composed mainly of the apical tuft dendrites of pyramidal neurons and afferent axons. This layer is virtually devoid of neurons; it has only a few local circuit neurons, and projection neurons are absent from layer I. Layer II has a granular appearance and is densely populated by the cell bodies of small pyramidal neurons. Layer III is composed of medium and large pyramidal neurons. The size of their cell bodies increases with the depth so that the largest layer III neurons are deep, near the border with layer IV. Layer IV has a granular appearance. It is composed of the small round cell bodies of stellate projection neurons and pyramidal neurons that do not have orienting apical dendrites. Often, the cell packing density in layer IV is the greatest of all the cortical layers. Layer V contains the largest pyramidal neurons. The cell packing density in this layer is the lowest of all neuron-rich cortical laminae. Layer VI has multiform projection neurons; that is, the cell bodies of layer VI neurons have many different shapes. Local circuit neurons are distributed in all cortical layers, however, their distribution does not facilitate the cytoarchitectonic differentiation of cortical layers and areas.

A comparison of somatosensory and motor cortices (Fig. 5) provides an example of how cortical areas can be differentiated on the basis of their cytoarchitecture. The superficial layers I to IV of somatosensory cortex contain many small neurons that endow it with a granular appearance. This granularity is particularly evident in layers II and IV. Somatosensory cortex also is characterized by relatively small layer V pyramidal neurons. In contrast, motor cortex has almost no layer IV and relatively few small neurons in layers II and III. Instead, there are many large pyramidal neurons, particularly in layer V. The largest layer V pyramidal neurons are called Betz cells, named after the 19th century

scientist that first described them. The Betz cells and other layer V projection neurons project axons to the spinal cord via the corticospinal tract. Although the pyramidal neurons in layer V of somatosensory cortex are smaller, some of them also contribute axons to the corticospinal tract.

Early in the 20th century, Karl Brodmann produced one of the most thorough and enduring cytoarchitectural studies of the human cerebral cortex. Figure 6 is a copy of the classic cytoarchitectural map that summarizes his conclusions about the distributions of cortical areas. Each cytoarchitectural area is designated with an Arabic numeral in the order in which he studied them. Accordingly, somatosensory cortex is area 3 (as described above) as well as adjacent areas 1 and 2. These areas are in the postcentral gyrus of the parietal lobe. Motor cortex is area 4 (as described above) and is in the precentral gyrus of the frontal lobe. Auditory cortex, areas 41 and 42, is located in the transverse gyri on the dorsal aspect of the superior temporal gyrus. Visual cortex, area 17 is found in the banks of the calcarine sulcus and the lateral wall of the occipital lobe. Other numbers used in this text refer to cortical areas designated by Brodmann.

Although most of the cytoarchitectonic areas on the lateral surface of the cerebral cortex are isocortical, many on the medial surface do not have six layers. The hippocampus, for example, has a pyramidal cell layer sandwiched between two plexiform layers. Cortical areas with fewer than six layers are referred to as *allocortex*. The allocortex is part of the anatomic limbic system.

Allocortex is very heterogeneous, however, and it is subdivided into three parts or moieties. (1) Archicortex includes the hippocampal formation (i.e., the hippocampus, dentate gyrus, and subiculum). (2) Paleocortex includes the olfactory piriform area and the rostral insula. (3) Periarchicortex includes many transitional or mesocortical areas such as entorhinal, posterior parahippocampal, and orbital cortices, as well as much of cingulate cortex. An example of the cytoarchitecture of some allocortical moieties are represented by drawings of neuronal perikarya in Fig. 7. The allocortical subiculum has a single pyramidal layer; the junction of this layer with the neuron-sparse molecular layer is indicated with an open arrow in this figure. Entorhinal cortex or Brodmann's area 28 is an example of periarchicortex and forms a major part of the parahippocampal gyrus. One of its prominent features are the islands of large star neurons in layer II; these neurons are surrounded by a

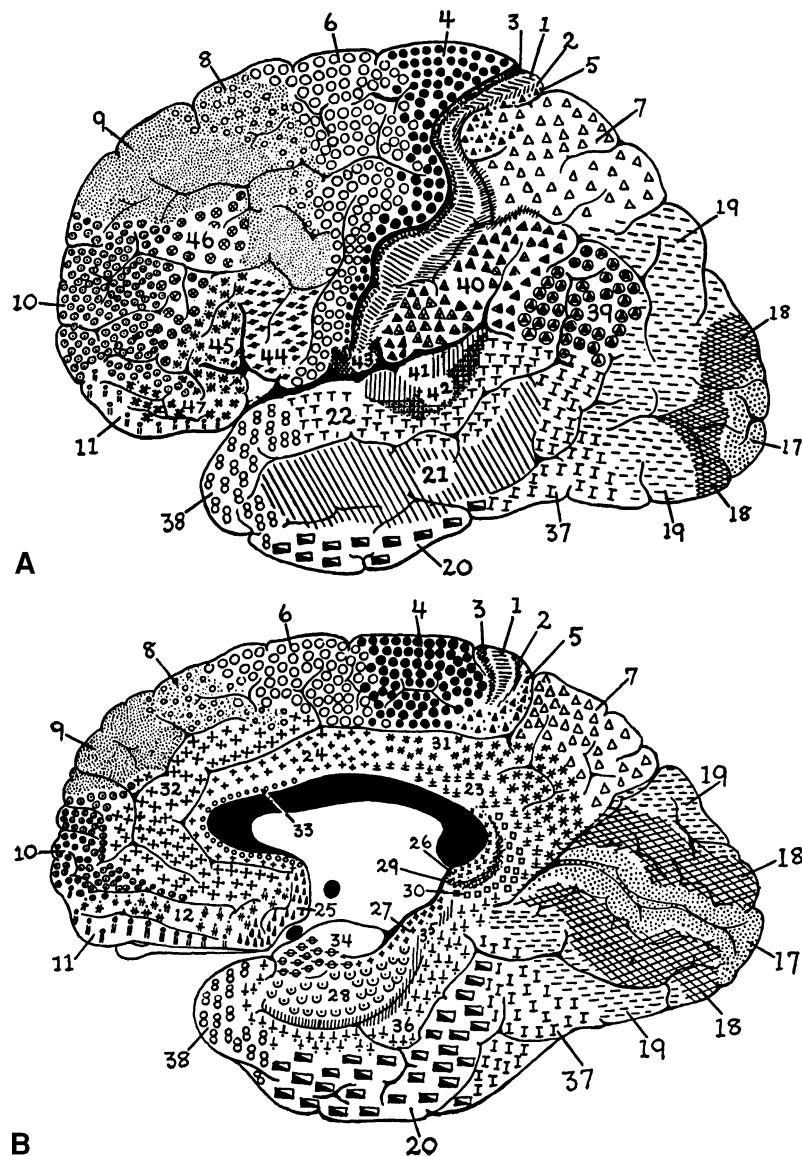


Fig. 6. Brodmann's maps of the distribution of cortical areas on the lateral (*top*) and medial (*bottom*) surfaces of the cerebral cortex.

dashed line in Fig. 7 to emphasize this “insular” arrangement. These neurons project into the hippocampal formation via the perforant pathway. Finally, neocortical area 20 forms much of the inferior temporal gyrus. This area has six layers including large layer III pyramidal neurons and granular layers II and IV.

One simple conceptualization of the cortical mantle is to view it as a sheet of layer V pyramidal neurons that is continuous from the hippocampus to isocortex. To this basic layer of neurons is added the superficial layer of pyramidal neurons that is differentiated in periarchicortical and isocortical areas to form the many cytoarchitectural variations that compose the cerebral cortex.

The various components of the cerebral cortex are affected differently in some neurologic and psychiatric diseases. This is exemplified by Alzheimer's disease. Figure 8 has examples of neurons from three regions of a brain from a person afflicted with Alzheimer's disease. The tissue is stained for neurofibrillary tangles with the fluorescent dye thioflavin S. Observe the flame-shaped neurofibrillary tangles that fill the perikarya of projection neurons. The structure of neurofibrillary tangle-laden projection neurons is shown for the hippocampal formation, layer II of entorhinal cortex, and layer III in isocortex. Neurofibrillary tangles also accumulate in layer V neurons of entorhinal and isocortical areas. The presence of this

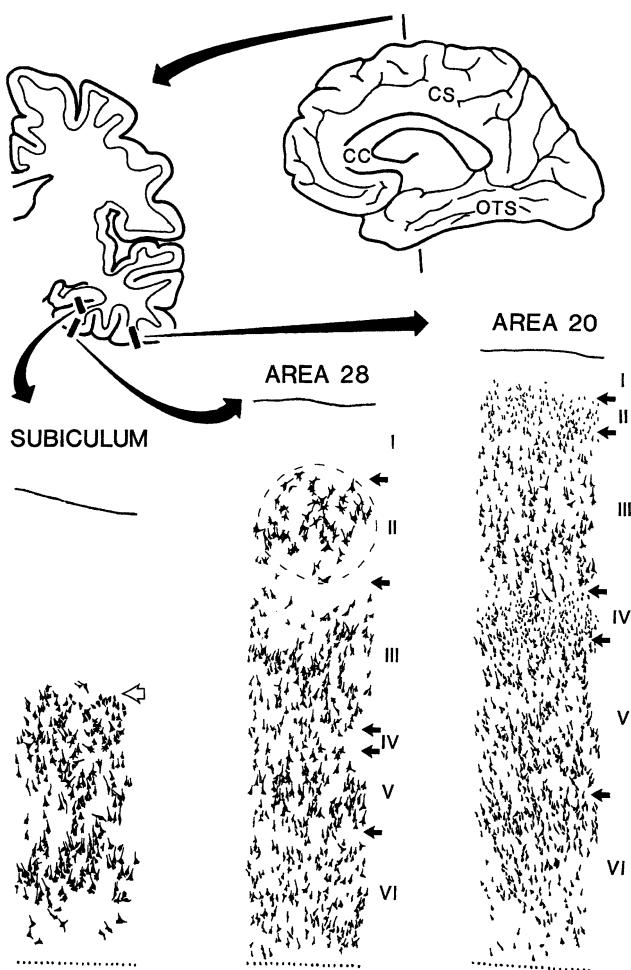


Fig. 7. The cerebral cortex is not cytoarchitecturally uniform and has a progressive elaboration in its architecture in the medial to lateral direction. This is true for both dorsal and ventral surfaces, although it is illustrated here with areas from the ventral part of the cortex. The medial view of the brain shows the corpus callosum (CC), cingulate sulcus (CS), and occipito-temporal sulcus (OTS) as well as the plane of section through which a coronal section was taken for Nissl staining. The top arrow indicates this section. The transverse section has three black rectangles through the subiculum, area 28, and area 20, respectively, in the medial to lateral direction. The three strips of cortex below are perikaryal drawings to show the distribution of neurons in each of these areas. The junction between the molecular and pyramidal cell layers of the subiculum is demarcated with an open arrow. In area 28, an island of layer II star pyramidal neurons is presented with a dashed line around it in order to emphasize that this is not a continuous layer of neurons as in most other cortical areas. Notice that each area in the medial to lateral direction has a progressively more differentiated laminar architecture.

pathology in large projection neurons is indicative of ongoing degenerative processes. In many instances, the somata completely disappear and only “ghost” neurofibrillary tangles remain. Because many of

these large neurons are corticocortical projection neurons, there is a disruption of major efferent projection systems of the cerebral cortex in this disease. Degeneration of these neurons, therefore, contributes to the cognitive and emotional impairments in Alzheimer’s disease.

5. FUNCTIONAL SUBDIVISIONS OF THE CEREBRAL CORTEX

Combined structural and functional studies led to systematic classification schemes of the principal functions of individual cortical areas. The main tools for functionally defining cortical areas are neuronal recording, electrical microstimulation, and positron emission tomography techniques.

5.1. Sensory Areas

Cortices involved in different aspects of sensation are subdivided into primary, first sensory association, second sensory association, and multimodal cortices. *Primary sensory cortices* contain neurons with spatially restricted receptive fields and properties that are dominated by thalamic afferents. For example, the lateral geniculate thalamic nucleus has neurons with receptive fields that are round and subtend as little as 1° of the visual field. Layer IV of primary visual area 17 (the lamina in which thalamic afferents principally arborize) has neurons with rectangular receptive fields the size and orientation of which are the product of multiple thalamic inputs terminating on individual neurons.

Sensory spaces can have *multiple representations* in the primary sensory cortices. In primary somatosensory cortex (SI), for example, there are four separate body representations. These separate sensory representations are contained within unique cytoarchitectural areas. Thus, primary somatosensory cortex is composed of areas 3a, 3b, 1, and 2, and each area has its own representation of the body surface. These body representations are termed *homunculi*. Each area is involved in the high-resolution localization and discrimination of somatic stimuli. Body regions with the highest density of receptors, such as the face and hands, also have the largest area of representation in the cortex, thus improving localization and discrimination of sensory stimuli in these areas. Each of the primary sensory areas are indicated with vertical lines in Fig. 9.

Parasensory association cortices receive their inputs mainly from the primary areas. Neuronal responses in these areas are more complex and involve the integration of a number of cortical inputs

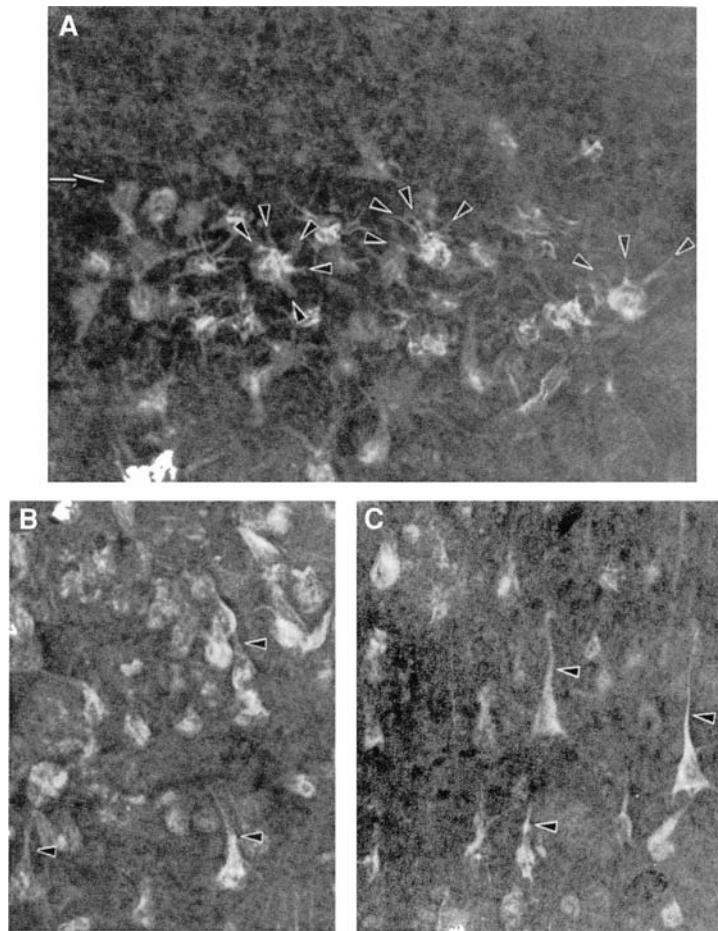


Fig. 8. Micrographs of thioflavin S-stained neurofibrillary tangles in (A) layer II of area 28, (B) the subiculum, and (C) layer III of area 20 of a case of Alzheimer's disease. Note in (B) and (C) that the apical dendrites of pyramidal neurons (arrowheads) are filled with these tangles as are the somata. The border of layers I and II of entorhinal cortex are marked with an arrow in (A). In this island of layer II neurons, there are almost no primary, or orienting, apical dendrites. Rather, neurons in this layer have dendrites that radiate from around the soma (arrowheads), and so these neurons are referred to as star cells.

as well as those from the thalamus. As shown in Fig. 9, each primary sensory area has a first parasensory association area: the first visual association area (VA1) for primary visual cortex includes areas 18 and 19, the first auditory association area (AA1) for primary auditory cortex includes part of area 22, and the first somatosensory association area (SA1) for primary somatosensory cortex includes area 5 and rostral area 40. In the left hemisphere, there is a specialization of the first auditory association area called Wernicke's area. Wernicke's area is a posterior part of AA1 and is involved in recognition of spoken language. It is important to note that layer III pyramidal neurons are the main source and target of corticocortical projections. Therefore, layer III neurons are particularly well developed in sensory association areas. Figure 5 shows the architecture of area 5 with its large layer III pyramidal neurons.

Secondary sensory association cortices are characterized by their corticocortical connections. The second sensory association areas receive inputs from the first sensory association cortices and project to multimodal areas. There is a second sensory association cortex adjacent to each of the first association areas; VA2 for visual cortex, AA2 for auditory cortex, and SA2 for somatosensory cortex (Fig. 9).

Multimodal association areas receive inputs from more than one sensory modality and so provide for intermodal associations among stimuli arriving in two or more second sensory association cortices. Thalamic nuclei that project to multimodal areas include the pulvinar, lateral posterior, and mediodorsal nuclei. These thalamic nuclei do not have a singular sensory function and are themselves likely sites for multimodal interactions. Multimodal areas can be classified into bi- and trimodal cortices. Although

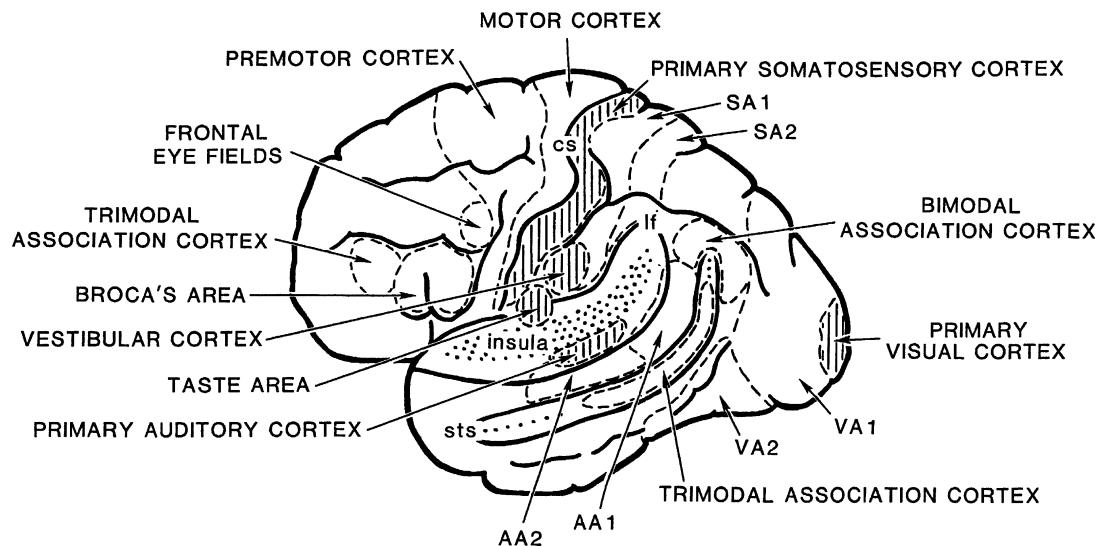


Fig. 9. Topographic distribution of functional areas in the cerebral cortex. These regions were determined from a wide range of physiologic and anatomic studies in human and non-human primate brains and so are highly schematic. They were plotted onto the same hemisphere as that used by Brodmann in the top of Fig. 6 so that functional regions can be compared with their associated cytoarchitectural areas. Primary sensory cortices are indicated with vertical hatching. A few sulci are labeled for orientation purposes and include the central sulcus (cs), lateral fissure (lf), and superior temporal sulcus (sts). Furthermore, the lateral fissure and superior temporal sulcus were “opened” so that the depths of these sulci can be visualized. By lifting the frontal, parietal, and temporal opercula, the underlying insula can be seen. The taste area extends onto the upper bank of the parietal operculum, whereas the primary auditory cortex is on Heschl’s gyri on the superior temporal plane (i.e., the planum temporale). The superior temporal sulcus was “opened” in order to expose one of the trimodal association cortices.

there are many bimodal areas, Fig. 9 presents only one as an example. This one is on the angular gyrus dorsal to the tip of the superior temporal sulcus, and it receives inputs from the second association areas for the visual and somatosensory modalities. There are three major trimodal association areas (two are shown in Fig. 9). One is in the ventral part of area 46 in prefrontal cortex, one is in the depths of the superior temporal sulcus, and a third is in posterior parahippocampal cortex on the ventral surface of the cerebral cortex.

Each multimodal association area has projections to cingulate and rostral parahippocampal cortices. These limbic areas are thought to be involved in monitoring the sensory environment because they have neurons that respond to large sensory stimuli, and lesions in cingulate cortex disrupt attention to sensory stimuli. Furthermore, ablations in parahippocampal, hippocampal, and cingulate cortices disrupt memory formation and spatial orientation. Thus, limbic cortices likely form an “end stage” in cortical processing of sensory inputs and provide mechanisms for memory that apply to complex patterns of sensory stimuli.

5.2. Motor Cortex

Activity in the various sensory spaces leads to environmentally adapted behaviors. The relationships between sensory and motor cortices and the mechanisms by which particular motor sequences are generated are prominent issues in clinical neuroscience. Motor cortices have direct projections to the spinal cord and/or brain-stem motor nuclei and are classified according to their contributions to movement based on responses to electrical microstimulation and the length of time by which neuronal firing precedes movement. *Primary motor cortex* (MI), Brodmann’s area 4 in the precentral gyrus, has the lowest threshold for electrically evoked movements. Thus, activation of neurons in motor cortex engages a limited number of muscle groups such as the lumbrical muscles for each finger. Neuronal activity in MI occurs just prior to movement. Thus, the finest control of motor activity is mediated by MI.

A dramatic example of the topographic representation of the musculature in motor cortex (i.e., the homunculus) is when seizure activity spreads through motor cortex in what is termed the *Jacksonian march*. When seizure activity is initiated in one part of the

homunculus, it induces convulsions in the associated part of the body. As the seizure activity spreads through the network of local axon collaterals of projection neurons, the large depolarizing shifts in projection neurons induces hyperexcitability in adjacent cortical areas. The result is convulsions that progressively include adjacent parts of the body. This seizure activity can spread across the entire motor cortex as well as via connections through the corpus callosum to the contralateral hemisphere. Thus, convulsions that originally start in one focus can spread across the body surface and involve both sides of the body.

Classic accounts of motor cortex defined a secondary or supplementary motor area (SMA) on the superior frontal gyrus medial to MI. SMA is one of a number of separate *premotor areas*. Neurons in premotor areas have direct projections to MI, the spinal cord, and/or brain-stem motor nuclei. Electrical microstimulation in these areas can activate larger groups of muscles; the movements elicited have a longer latency than those resulting from stimulation of units in primary motor cortex. One of the premotor areas directs eye movements and is shown in Fig. 5 as the frontal eye fields. Another area controls movements of the mouth during speech and is termed *Broca's area*. Broca's area is directly connected to Wernicke's area, which is part of the first auditory association cortex in the left hemisphere. Thus, language comprehension in Wernicke's area can control speech via Broca's area.

The classic studies of epileptic patients by the neurosurgeons Wilder Penfield and Herbert Jasper shaped our understanding of the functional organization of the cerebral cortex. They surgically exposed the cerebral cortex under general anesthesia and then allowed the patient to regain consciousness so that the patient's sensations could be reported after electrical stimulation or during seizure activity. In addition to observing sensory and motor phenomena after electrical stimulation of sensory and motor cortices, respectively, Penfield and Jasper observed that stimulation of limbic cortical areas evoked visceral and emotional sensations and memories. Case N.C., for example, had seizure activity that began with a "far-off" feeling. She smacked her lips, swallowed, and complained of nausea. Borborygmi could be heard from her abdomen during the seizure, and these attacks were often associated with a feeling of panic. Electrical stimulation of the anterior temporal lobe and rostral insula evoked similar feelings and intestinal activity, and ablation of these cortices alleviated the seizure activity and associated emotions

and autonomic activity. Thus, limbic cortex contributes to visceromotor activity and emotion as well as operating as an end stage in sensory processing as noted above. These limbic areas include orbital, insular, temporal pole, and anterior cingulate cortices.

6. CORTICAL CONNECTIVITY

6.1. Thalamocortical Relationships and the Cortical Column

One of the principles of neuroscience is that the activity in sensory thalamic afferents defines the primary functions of a sensory cortical area. Thus, as noted above, the receptive field characteristics of neurons in the lateral geniculate nucleus determine the visual properties of neurons in area 17. The thalamic projections terminate chiefly in layer IV with minor projections to layers I and VI. As a result, the thalamic afferents synapse with any element (cell body or dendrite) that compose these layers (regardless of where the cell body is located). It is also the case that projections from the ventrolateral and ventroanterior thalamic nuclei contribute to the motor functions of motor and premotor areas, respectively. The pivotal role of the thalamic input is confirmed by transplantation studies. After transplantation of fetal somatosensory cortex to visual cortex, the neurons from somatosensory cortex respond to visual stimuli.

The termination of thalamic afferent axons in the cerebral cortex determine functional cortical modules or columns. Vernon Mountcastle and his colleagues first showed that neurons in a vertical column of somatosensory cortex shared similar response properties. Thus, one column contains neurons with preferential responses to stimulation of joints and deep tissues, whereas an adjacent column might contain neurons with preferential responses to light touch of the skin. The columns in visual cortex represent ocular dominance and orientation specificity (Fig. 10). In sensory cortices, these columns measure 500 to 1000 μm in diameter and reflect the distribution of thalamic axon terminals in the cortex.

Aggregates of neurons form columns in motor cortex. Unlike the columns in sensory cortices, the columns of functionally similar neurons are assessed in terms of the output of motor cortex (i.e., by microstimulation techniques rather than receptive field mapping). The output columns in motor cortex are larger than in sensory cortices being up to 2 mm in diameter. These large sizes are due to the size of dendritic fields of particularly large pyramidal neurons in motor cortex and are specified according to the amount of cortex

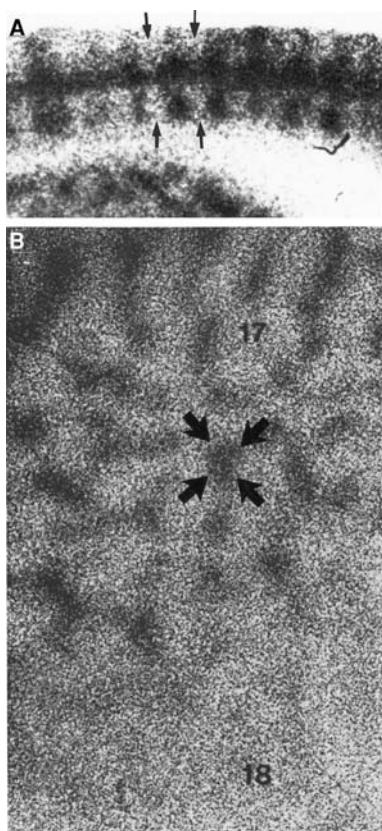


Fig. 10. (A) Presentation of a bar of light with a particular orientation results in partial stimulation of visual cortex. The responsive neurons in area 17 are organized in vertical, or radial columns (arrows). These columns were visualized autoradiographically using 2-[¹⁴C]deoxyglucose, radioactive tracer for glucose. This tracer was taken up and trapped in metabolically active neurons. The highest activity being in layer IV, the termination site of the thalamic afferents. (Reproduced with permission, Hubel, Weisel, and Stryker, J. Comp. Neurol. 1978;177:361–380). (B) An autoradiograph of a tangential section through superficial cortex reveals a complex patchwork of activity. Note that the orientation specific columns (arrows) are present only in area 17 but not in visual association cortex, area 18. (Reproduced with permission, Gilbert and Weisel, J. Neurosci. 1989;9:2432–2442.)

that must be electrically stimulated to evoke contraction in a small group of muscles.

Thalamocortical connections are *reciprocal*. That is, a column of neurons defined by a particular thalamic input to layer IV contains pyramidal neurons in layer VI that project to the thalamus. These return projections are organized in a point-to-point fashion so that, for example, cortical points representing the central visual fields project to similar places in the lateral geniculate nucleus. These reciprocal connections are thought to be involved in modulating the receptive field properties of thalamic neurons.

6.2. Monoaminergic Afferents and Sleep

The cerebral cortex is innervated by two ascending monoaminergic afferent systems. The noradrenergic and serotonergic systems arise from brain-stem nuclei and project throughout the ipsilateral hemisphere. These projections are widely distributed and cross cytoarchitectonic borders.

Noradrenergic axons project from neuronal somata in pontine and mesencephalic nuclei. The most notable of these nuclei is the locus coeruleus, so named because it appears as a blue site in the fresh brain. This is a small, pigmented nucleus in the floor of the fourth ventricle. The noradrenergic afferents terminate in all layers of cortex, but predominately in layers I to IV. The cortical terminals of the noradrenergic afferents contain dense core vesicles. These vesicles package the norepinephrine prior to its exocytotic release in the synaptic cleft where the norepinephrine binds with α - and β -adrenoceptors. The laminar distribution of these receptors differs. α_1 -Adrenoceptors are distributed in layers I to IV, whereas α_2 -adrenoceptors are most common in layers I and IV. β -Adrenoceptors are most densely distributed in layers I to III. The interaction of norepinephrine with these receptors produces different responses. For example, activation of α_2 -adrenoceptors (which presumably are on the presynaptic membrane) opens potassium channels, inhibiting further release of norepinephrine. In contrast, binding of norepinephrine with the α_1 - and β -adrenoceptors closes potassium channels in postsynaptic neurons and leads to the excitation of these neurons.

The serotonergic afferents arise from the dorsal and median raphe nuclei. These are small clusters of neurons that are located along the midline of the pons and caudal midbrain. Interestingly, raphe neurons also colocalize neuroactive peptides such as substance P, *leu*-enkephalin, and thyrotropin-releasing hormone. Serotonergic afferents innervate layers I, III, and IV. Likewise, the cortical serotonin receptors are largely confined to layers III and IV. The release of serotonin results in changes in the permeability of potassium channels, which in turn leads to complex modulation of the activity of postsynaptic neurons.

The monoaminergic afferents to cortex are thought to be involved in the regulation of sleep and arousal. Serotonergic neurons in the dorsal raphe exhibit a slow, rhythmic activity. The pacemaker activity is modulated by the activity of noradrenergic afferents. This pacemaker activity is accelerated by the closing of potassium channels by norepinephrine. The activity of the pacemaker neurons changes with

the state of arousal; being highest when the person is awake, lower during periods of “slow-wave” sleep, and lowest during periods of dream (or rapid eye movement; REM) sleep. The activity of noradrenergic raphe neurons rises during slow-wave sleep and falls during periods of REM. The phasic activity of a population of cholinergic neurons in the reticular formation (the gigantocellular tegmental field) rises during REM-sleep. Interestingly, these neurons are innervated by descending cortical projections, which serve in a feedback capacity. Thus, it appears that an interaction of a number of neurotransmitter systems regulates the states of consciousness.

6.3. Cortical Cholinergic Connections and Memory

Memory formation is a process that involves short-term and long-term events as well as many cortical areas and a number of cortical afferents. It is beyond the scope of this chapter to detail what is known about the mechanisms of memory. A thumbnail sketch, however, should note the following observations. The hippocampus is critical for short-term memory, whereas long-term memories are likely stored in many of the multimodal association and limbic cortices. Because acetylcholine receptor antagonists interfere with memory formation, it is thought that cholinergic afferents are important for the operation of cortical circuits involved in memory formation. It is possible, for example, that cholinergic connections operate as an “enabling switch” that allows particular memories to be transferred from short-term to long-term storage.

There are two sources of acetylcholine in the cerebral cortex. About 70% originates in neurons with cell bodies in the magnocellular basal forebrain nuclei, and 30% arises from a subpopulation of cortical local circuit neurons. The basal forebrain nuclei include the medial septum, the basal nucleus of Meynert, the diagonal band of Broca, and the substantia innominata. Medial septal neurons project to the hippocampus. Projections from neurons in the basal nucleus of Meynert and medial segments of the diagonal band of Broca terminate in medial cortical areas including entorhinal, medial prefrontal, and cingulate cortices. Neurons in the substantia innominata and lateral segments of the diagonal band of Broca project to many neocortical areas including temporal, parietal, and occipital neocortical areas. Projections to the cerebral cortex generally terminate in layers V and VI, although there are projections to the superficial layers.

Disruption of cholinergic connections may contribute to early signs of memory loss and spatial disorientation observed in patients with Alzheimer’s disease. Neurofibrillary tangles and neuronal degeneration occur in the basal forebrain nuclei and are considered to be pathologic hallmarks of Alzheimer’s disease. Memory deficits in Alzheimer’s patients are associated with damage (1) to the hippocampus, which is engaged during short-term memory formation, (2) to multimodal and periarchicortical limbic areas involved in long-term memory storage, and (3) to the cholinergic system, which may be involved in enabling these structures to convert short-term to long-term memories.

6.4. Neurotrophin Systems

The family of neurotrophins includes nerve growth factor (NGF), brain-derived growth factor (BDNF), and neurotrophin 3 (NT-3). Neurons throughout the depth of cortex express the neurotrophins; indeed, many coexpress multiple neurotrophins. Alterations of cortical neurotrophins lead to deficits in neural plasticity and in learning and memory. For example, the brains of people with Alzheimer’s disease or of people who have chronically abused alcohol are characterized by dysregulated neurotrophins.

Neurotrophins bind to specific receptors. One set of receptors binds neurotrophins with high affinity. An active part of the intracellular portion of these receptors is a tyrosine kinase, hence, they are known as trk’s. Three trk’s have been identified, trkB, trkC; they differ in their extracellular domains and have high preference for nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin 3(NT-3), respectively. Another receptor, p75, binds neurotrophins with lower affinity. All of the neurotrophin receptors are highly expressed in cortex, especially by layer V pyramidal neurons.

A neurotrophin can be released by cortical neurons at synaptic sites, taken up by cholinergic axons expressing trkB, and retrogradely transported back to the basal forebrain. Using such a retrograde system, cortical neurons are considered to be invaluable for supporting basal forebrain cholinergic projections, which in turn are important in gating cortical processing (see above). Interestingly, neurotrophin receptors are most commonly exhibited at dendritic and somatic synaptic sites. Only few axons express neurotrophin receptors. Thus, it appears that neurotrophins can act through systems that parallel neurotransmission systems, that is, autocrine and paracrine mechanisms as well as anterograde systems. This implies that the role of neurotrophins in learning and memory primarily is a product of intracortical processing.

6.5. Interhemispheric Connections and the Unification of Cognitive Activity

Each hemisphere contains the representation of the contralateral sensory field. Although there is slight overlap at the midline, such as in the region of central vision and the somatotopic representation of the trunk, the separation of the two perceptual spaces is rather clean. Despite this division, each person perceives a seamless representation of the world. The coordination of the processing that goes on in each hemisphere depends upon axons that cross the *corpus callosum*.

Many cortical areas on one side of the brain are connected with areas in the contralateral hemisphere via callosal connections. Although the details about the callosal system vary among discrete cytoarchitectonic areas, some general patterns can be described. The axons of the callosal pathway arise from pyramidal neurons in layer III and to a lesser extent from layer V projection neurons. These axons descend into the white matter and pass across the corpus callosum. After entering the contralateral hemisphere, the axons follow a mirror image course and terminate in layers I, III, and IV of the homotopic, or corresponding, site in the contralateral hemisphere.

The function of callosal connections can be gleaned from studies by Roger Sperry and colleagues who examined people whose corpora callosa and anterior commissures were transected. Overtly, such people with “split” brains operate perfectly well, but when challenged with certain tasks, it is clear that each hemisphere is not capable of executing all tasks. In one experiment, a split-brain subject was presented with an apple in his or her right or left visual fields. After presentation to the right visual field, a subject was able to verbally describe the object. In contrast, when the apple was placed in the left visual field, the person was unable to come up with the word *apple*, but he or she was able to point to an image of the apple when offered a choice of images. This experiment shows that both hemispheres receive sensory information but differ in their communicative capabilities. The functional separation of abilities characterizes some of the hemispheric asymmetries. Although it is difficult to make generalizations as to the functions of each hemisphere, each hemisphere is best able to mediate either verbal or nonverbal communication. At the risk of oversimplifying the situation, it appears that the left hemisphere is expressive and best at analytical, rational thought, whereas the right hemisphere is perceptive and capable of emotional, intuitive thought.

The lateralization of function is expressed in normal, intact people as cerebral dominance in language and handedness. Virtually all right-handed people have dominant left hemispheres. As might be expected, a significant percentage (15%) of left- or mixed-handed people express right hemispheric dominance, but interestingly, the dominant hemisphere for language in most of these people (70%) is the left hemisphere. Thus, regardless of the outward expression of handedness, the left hemisphere is usually the dominant hemisphere for language.

6.6. Projections to Nonthalamic Subcortical Systems

In addition to the corticospinal (pyramidal) pathway (mentioned above), the axons of layer V neurons project to extrapyramidal targets as summarized in Fig. 11. These targets are sensory, motor, and integrative centers. Cortical afferents innervate *sensory* cranial nerve nuclei such as the trigeminal brain-stem nuclear complex and the solitary nucleus. These projections serve as feedback controls modulating the output from second-order sensory nuclei. Other descending axons project to *motor* centers. These targets include motor cranial nerve nuclei that have direct projections to skeletal muscle. In specific, these nuclei are the trigeminal motor (cranial nerve [CN] V), facial (CN VII), supraspinal (CN XI), and hypoglossal nuclei (CN XII). These corticobulbar projections serve as upper motor neurons analogous to the corticospinal projections. In addition, cerebral cortex projects to three structures that in turn project to the spinal cord. (1) Projections from frontal, parietal, occipital, and temporal lobes project to the superior colliculus. These afferents carry somatosensory, visual, and auditory information that enables the superior colliculus to execute its role in coordinating complex behaviors such as tracking and attending to moving stimuli. (2) Cortical afferents, primarily from the precentral gyrus, synapse with neurons throughout the red nuclei. (3) Two nuclei in the reticular formation receive cortical input, the gigantocellular region of the medullary reticular formation and the oral area of the pontine reticular formation. Extrapyramidal projections from layer V neurons also terminate in nuclei that are *integrative* centers. These targets include the caudate nucleus, the periaqueductal gray, and the pontine nuclei.

The cortical projections that innervate brain-stem structures follow a pathway that is common with the corticospinal tract. That is, the cortical projections

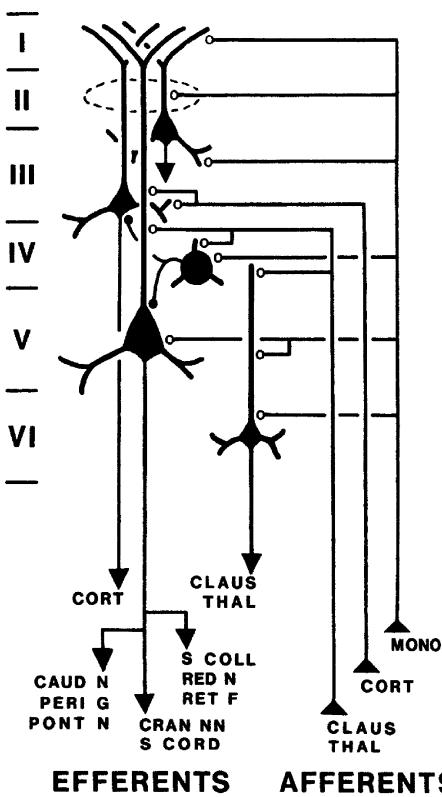


Fig. 11. A summary diagram of the intrinsic organization and connections of a “generic” neocortical area. Three pyramidal neurons forming a cluster (within the dashed lines) are shown. Excitatory connections are indicated with *open axon terminals*, whereas inhibitory ones are shown with *solid axon terminals*. Abbreviations for these connections are as follows: caud n, caudate nucleus; claus, claustrum; cort, corticocortical connections for both ipsilateral and contralateral hemispheres; cran nn, sensory and motor cranial nerve nuclei; mono, monoamines including norepinephrine and serotonin; peri g, periaqueductal gray; pont n, pontine nuclei; red n, red nucleus; ret f, reticular formation; s coll, superior colliculus; s cord, spinal cord; thal, thalamus.

pass through the subcortical white matter, the internal capsules, the crus cerebri, the base of the pons, and the pyramidal tracts. The only difference is that the axons exit from the common pathway at the level of the structure being innervated. For example, corticobulbar fibers that innervate the hypoglossal nuclei descend with the corticospinal neurons, but perpendicular arbors leave the pyramidal tract in the caudal medulla. Thus, the size of the descending common pathway decreases as the axons exit.

Cortex is interconnected with the *claustrum*. The claustrum is a small sheet of neurons embedded in the subcortical white matter. The corticoclastral interconnections largely parallel the corticothalamic interconnections. The claustrum sends cortical afferents

to layer IV and to a lesser extent to layer VI. Cortex in turn projects to claustrum; these projections originate in layer VI neurons in many cortical areas including primary auditory, somatosensory, and visual cortices. The function of the claustrocortical relationship largely remains a mystery, however, it may play a role in the modulation of sensory receptive field properties. For example, claustral afferents to visual cortex contribute to the property of end-inhibition, whereby the sizes of certain receptive fields are limited by antagonistic effects. The corticoclastral connections, like the corticothalamic projections, probably feed back on the claustrum to modulate the activity of the claustral neurons. These reciprocal projections may also provide a system for the integration of the three sensory spaces within the claustrum.

7. SUMMARY OF THE ORGANIZATION OF THE CEREBRAL CORTEX

The organization of the cerebral cortex from the perspectives of intrinsic circuitry and afferent and efferent connections is summarized in Fig. 11. It is built around a small group of layer III and V neurons whose apical dendrites are aggregated into a cluster. Afferent connections are shown to the right and include excitatory thalamic afferents to layer IV, excitatory cortical connections from ipsilateral and contralateral cortices to layer III, and a diffuse distribution of serotonergic and noradrenergic projections that innervate elements in all cortical laminae. A single local circuit neuron is shown in layer IV with its inhibitory connections with pyramidal neurons, however, it should be remembered that these neurons are diffusely distributed throughout all layers of the cortex.

Cortical neurons project to a number of extrinsic sites. The actual density of any one of these projections depends upon the specific cortical area under consideration. All cortical areas have ipsilateral cortical projections that originate in layer III and projections to the thalamus, pontine, and caudate nuclei that originate from layer V neurons. Figure 11 does not intend to imply that each layer V pyramidal neuron makes all of these projections. It is likely that a single pyramidal neuron in layer V has axon collaterals that project to one to three subcortical sites. Specialization in projection systems for each cortical area occurs in terms of inputs to the superior colliculus, reticular formation, sensory and motor cranial nerve nuclei, and the red nucleus. Many of these connections are unique to parts of particular motor and premotor areas.

Sensory and motor information interact in cortex; visual, auditory, and somatosensory information can be integrated, stored, and recalled so that the appropriate motor system(s) can be activated. All of these processes are essential for mnemonic mechanisms and cognition. In fact, cortex is pivotal for all of the processing that underlies the complex thought and communication considered to be uniquely human. Nevertheless, it cannot be emphasized enough that the cerebral cortex does not act in isolation. Cortex can function only because of the diverse interconnections with other CNS structures.

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Dementia and Abnormalities of Cognition

Gregory Cooper, Gerald Eichhorn and Robert Rodnitzky

Dementia refers to a progressive impairment involving multiple spheres of cognition sufficient to prevent the normal accomplishment of standard activities of daily living such as dressing, cooking a meal, or balancing a checkbook. Less severe impairment that does not result in a loss of ability to perform normal activities of daily living is often termed mild cognitive impairment (MCI). Although there may be a mild decline in cognitive function that occurs with normal aging, this would not be expected to lead to serious functional disability.

REGIONAL BRAIN PATHOLOGY

The individual signs and symptoms of dementia reflect the underlying regional brain pathology. For example, the clinical characteristics of dementia occurring in diseases affecting primarily the cerebral cortex differ from those of subcortical dementia. In cortical disorders such as Alzheimer's disease, symptoms specific to cortical dysfunction include aphasia, suggesting involvement of perisylvian cortices, apraxia (e.g., inability to organize and perform a motor task despite normal strength and coordination), and agnosia (e.g., inability to recognize), suggesting temporal and parietal cortical involvement. Visuospatial difficulties indicate the involvement of parietal cortices, and impairments in visual processing suggest involvement of visual-association cortices. In other conditions affecting frontal cortices, marked alterations in personality and behavior may be seen. In dementias primarily related to subcortical pathology, such as that occurring in Parkinson's disease, these features are usually absent. In both forms,

memory loss occurs, but it is typically less severe in subcortical dementia. The memory loss associated with subcortical dementia is frequently amenable to cuing, and usually a hint will bring the forgotten thought to mind. It has been said that those with cortical dementias forget, and those with subcortical dementia, who can recall after being cued, forget to remember. In other words, memory loss in cortical dementia reflects impaired learning and in subcortical dementia impaired recall. Subcortical dementia is also marked by a general cognitive slowing, sometimes termed *bradyphrenia*.

ALZHEIMER'S DISEASE

Alzheimer's Disease Is the Most Common Cause of Dementia

Although more than 70 causes of dementia have been identified, Alzheimer's disease is responsible for 60% or more of cases of dementia in persons older than 65 years of age. This condition is characterized pathologically by the presence of neurofibrillary tangles and senile plaques within the brains of affected individuals. Both of these microscopic abnormalities are predominately found in the neocortical areas and the hippocampus. Neurofibrillary tangles result from the accumulation of hyperphosphorylated tau protein within neurons, whereas senile plaques consist of degenerating neuritic processes surrounding an amyloid core. This core is composed of aggregated β -amyloid protein ($A\beta$), which is derived from the larger amyloid precursor protein (APP). $A\beta$ is formed when APP is cleaved by β - and γ -secretase. The exact function of the APP has not been resolved.

However, it has been suggested that A β , possibly through direct neuronal toxicity, is responsible for the cascade of events leading to the signs of symptoms of Alzheimer's disease. Support for the amyloid hypothesis comes from findings in cases of familial Alzheimer's disease. Mutations in the APP-encoding gene on chromosome 21 and in the presenilin genes on chromosomes 1 and 14 all lead to increased production of A β . In addition, the $\epsilon 4$ allele of the APOE gene has been identified as a risk factor for Alzheimer's disease in a dose-dependent fashion. It has been proposed that this risk is mediated through alterations in A β metabolism, and it has been shown that subjects with one or more $\epsilon 4$ alleles have higher amyloid plaque burdens. These findings have led to considerable interest in developing agents that will alter APP metabolism or promote clearance of A β .

Memory Loss Is the Most Constant Feature of Alzheimer's Disease

The clinical hallmark of Alzheimer's disease is memory loss. Recent memory is affected initially, reflecting impaired learning. This may manifest as repetitive questioning, missed appointments, or misplaced objects. In contrast, memory of more remote events is often relatively preserved in the early stages. Individuals with mild Alzheimer's disease are often described as "living in the past," which is likely a reflection of their impaired recent memory and relatively intact remote memory. The onset of memory loss is insidious, and impairment is slowly progressive. In Alzheimer's disease, memory loss may in part be related to neuronal loss in the hippocampus and a reduction in the number of cholinergic neurons projecting from the basal nucleus of Meynert in the forebrain. Attempts to augment or restore cholinergic transmission through the use of cholinesterase inhibitors have proved to be modestly effective in improving memory and cognition in Alzheimer's disease. Glutamatergic dysfunction has also been hypothesized to be a contributing factor in Alzheimer's disease and has led to the more recent addition of an NMDA antagonist (memantine) to the pharmacologic armamentarium against Alzheimer's disease.

SUDDEN ABNORMALITIES OF MEMORY AND COGNITION

Sudden abnormalities of memory and cognition are more likely caused by brain tumors or stroke than by degenerative conditions. Symptoms similar

to those seen in Alzheimer's disease can occur as a result of localized damage to specific regions of the brain from a brain tumor or cerebral ischemia. Transient global amnesia (TGA) is the sudden but temporary inability to encode new memories, usually lasting a few hours. Affected patients remain alert but repeatedly ask the same orienting questions, because they cannot retain the information provided in previous answers. The episode resolves spontaneously, and without sequelae, though there is typically no recall of events during the episode of TGA. The etiology of TGA remains unclear. Though a vascular cause has been postulated, patients with TGA do not appear to be at increased risk for subsequent stroke or TIA. A spreading neuronal depression affecting the hippocampi has also been postulated as a cause for TGA.

Brain tumors or stroke involving the right parietal lobe often result in agnosia, an inability to recognize. Autopagnosia refers to the inability to recognize a part of one's own body. After a right parietal stroke, a person with autopagnosia may identify his or her own left hand as belonging to the examiner instead. Prosopagnosia is a specific agnosia that can result from highly localized cortical damage. It is characterized by the inability to recognize and identify familiar faces. This condition occurs in persons who have suffered damage to the visual-association cortices in both hemispheres.

Apraxia is the inability to perform skilled motor movements despite normal coordination and preserved motor, sensory, and cognitive function. The apractic patient may properly grasp and lift a spoon, but he cannot demonstrate how it is used. Apraxia most commonly occurs after damage to the inferior parietal region of the dominant hemisphere. Certain forms of apraxia are more related to impaired perception of spatial relations and are caused by lesions of the nondominant hemisphere. Dressing apraxia is an example of this phenomenon. The affected person cannot correctly perform acts such as putting an arm into the sleeve of a coat, buttoning buttons, or placing a garment on the correct part of the body.

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**Thomas van Groen, Inga Kadish, Lawrence Ver Hoef
and J. Michael Wyss**

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1. HISTORY OF THE NEUROANATOMY OF THE LIMBIC SYSTEM

The history of the anatomic term *limbic* for brain regions is quite extensive; Willis (1664) was the first to name the cortical regions that form the medial edge of the telencephalon as *limbus* (i.e., “border” in Latin), whereas Broca (1878) was the first to use the designation of this area of cortex as “Le grand lobe limbique,” or “the great limbic lobe.” Broca’s great limbic lobe comprised the gray matter areas that lay as the transition between the neocortex and the diencephalon (i.e., the cingulate, hippocampal, olfactory, and parahippocampal cortices) and that together formed a circle on the medial edge of the hemisphere (Fig. 1). The limbic lobe is rostrally connected to the olfactory bulb and is smaller in microsmatic animals and bigger in macrosomatic animals; this observation led Broca to suggest that the main function of the limbic lobe was related to olfaction. Later, several researchers expanded on this

view even to the point of suggesting that the limbic lobe was a “smell brain” or *rhinencephalon*. By the 1940s, several lines of evidence suggested that these regions of limbic cortex also received other types of sensory information and were possibly involved in other functions, especially emotion.

In 1937, Papez hypothesized that there was an anatomic basis for emotions. Based on the existing anatomic knowledge and on earlier proposals on the anatomic basis of emotional functioning, he proposed an anatomic circuit that showed how emotional experiences would lead to the expression of emotions. The circuit is now known as the *Papez circuit* (Fig. 2), which consists of a “circuit” of connections between limbic cortical areas and the diencephalon. Papez’s original hypothesis was that the cingulate cortices together with the hippocampal formation received a major input from sensory areas of the cerebral cortex and that the hippocampal formation processed this information and projected this to the mammillary bodies (hypothalamus) from which the appropriate emotional response could be coordinated. This circuitry has been elucidated in more detail, and it is now clear that the afferent

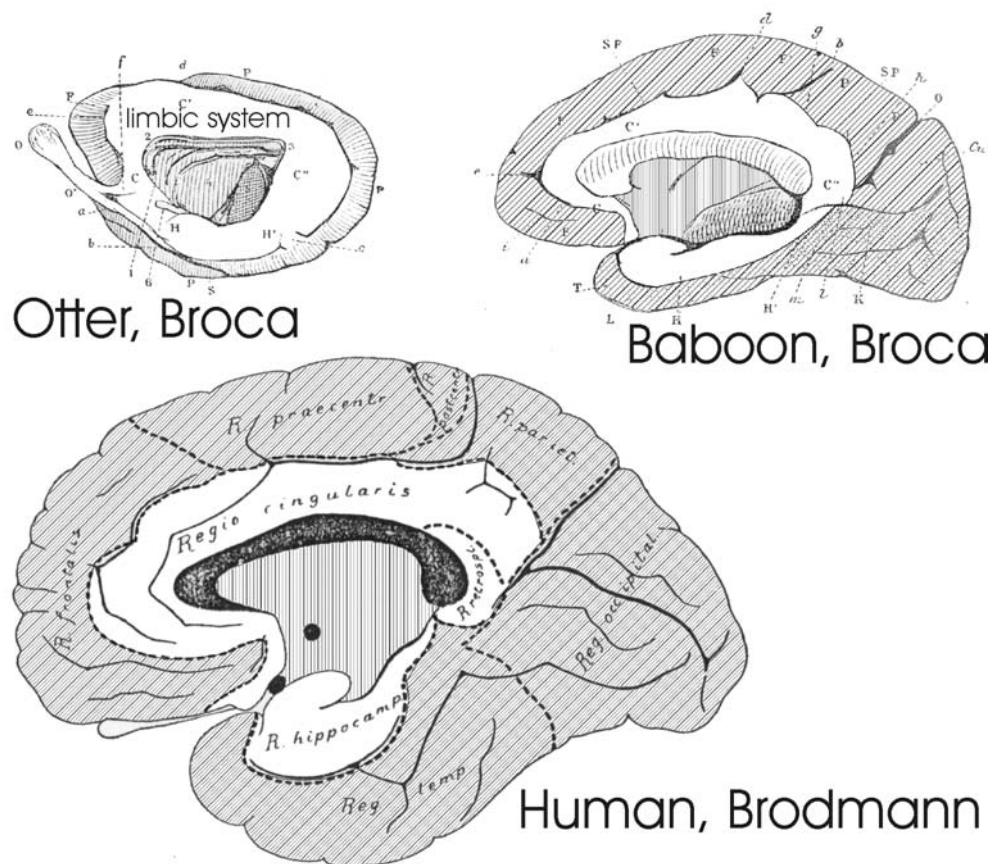


Fig. 1. In comparison with other animals, the limbic cortex, which encircles the upper brain stem, reaches its largest extent in human. These drawings of the medial surface of a hemisected brain, with brain stem and cerebellum removed, show the size and position of the limbic cortex in two mammals as illustrated by Broca and in human (modified from Brodmann).

and efferent connections of the hippocampal formation are far more varied and complex than Papez's original model (Fig. 2).

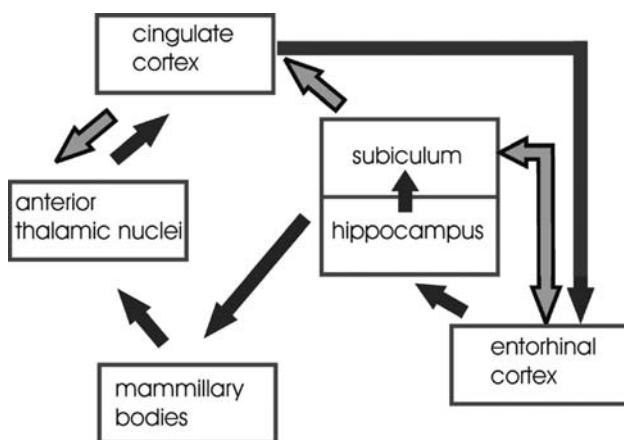


Fig. 2. Papez's circuit as originally proposed (black arrows) with additional connections now commonly considered important (gray arrows). In Papez's original work, the hippocampal formation was viewed as a single area.

Two years after Papez's hypothesis, Klüver and Bucy demonstrated that extensive lesions to the temporal lobe that primarily damage two parts of the limbic lobe (the amygdala and the hippocampal formation) profoundly influenced the affective behavior of sub-human primates. On the basis of Klüver and Bucy's and other studies, MacLean (1952) suggested that the term *limbic system* should be applied to the limbic areas. MacLean emphasized that the limbic system elements (which now also included the amygdala) were at the interface between somatic and visceral areas of the brain. The limbic system could relate these two systems to each other and to the ongoing behavior of the animal, accordingly he envisioned the limbic system functioning as a "visceral brain." Nauta further expanded on the definition of the limbic system by showing that areas of the limbic cortex were directly connected to many areas of the hypothalamus and brain stem. In this manner, the limbic system is a concept that has undergone considerable redefinition since its original introduction as "limbus."

2. NEUROANATOMY OF THE LIMBIC SYSTEM

The primary cortical areas that we currently include under the umbrella of limbic system are *allocortical areas* (i.e., olfactory cortex, basolateral amygdala, and hippocampal formation, including the subicular cortices) and *transitional areas between allocortex and isocortex* (i.e., nearly all parahippocampal cortex and cingulate cortex, but also caudal orbital and medial prefrontal cortex and part of the temporal polar cortex, and the ventral part of the agranular and dysgranular part of the insular cortex). All these regions are telencephalic, but they are structurally more simple than the isocortex (i.e., neocortex). Our concept of the limbic system is based on cortical developmental principles, in the sense that it contains nearly all non-isocortical parts of the cerebral hemisphere (i.e., allocortex and transitional cortex). *Telencephalic subcortical areas*, such as the cortical and central amygdala, the septal nuclei, and *diencephalic* regions, including the mammillary bodies and the anterior thalamic nuclei, make up the rest of the limbic system.

The limbic system areas are highly interconnected, both by direct connections and by indirect projections through diencephalic regions such as the mammillary bodies and the anterior thalamic nuclei. Together, these areas of the telencephalon and diencephalon and the myelinated axon bundles that interconnect these areas (i.e., fornix, mamillothalamic tract, stria terminalis, and stria medullaris thalami) form the limbic system (Fig. 3).

Although the individual limbic areas are functionally diverse, serving emotional responses on the one hand and learning and memory on the other, the high degree of interconnectivity suggests that these areas likely do have an underlying unity.

Despite the fact that our concept of the limbic system is based on cortical developmental principles (i.e., it consists primarily of relatively “simple” allocortex and transitional cortex), it is not a “primitive” part of the brain. Notwithstanding their phylogenetically early appearance (e.g., hippocampus = archicortex; ancient cortex), many limbic areas of cortex reach their greatest development in human, for instance, compared with primary sensory and motor cortices, the entorhinal cortex expands more in size from lower mammals to humans (Fig. 1). Accordingly, these areas should not be seen as antiquated remnants, but rather they are brain regions that have continued to develop both structurally and functionally throughout phylogeny.

Researchers may still differ as to which areas exactly comprise the limbic system; however, most

agree that it includes the *hippocampus*, *subicular cortex*, *parahippocampal cortex*, *cingulate cortex*, *septal nuclei*, *amygdala*, *mammillary bodies*, the anterior thalamic nuclei and their connections.

2.1. The Hippocampus Is a Major Landmark in the Temporal Cortex

2.1.1. OVERALL STRUCTURE

The hippocampus, or hippocampal formation, lies in the temporal lobe of the cerebral cortex, just medial to the inferior horn of the lateral ventricle. Some early anatomists thought its gross appearance looked like a seahorse and gave it the name *hippocampus* (originating from *hippokampos*, the Greek word for “seahorse”). The hippocampus stretches from the amygdala to the splenium of the corpus callosum, but the intrinsic structure of the hippocampus proper is best appreciated in its middle one third. In this region, the sheet of hippocampal tissue is folded over itself in its characteristic S-shape (Fig. 4). In more rostral regions of the hippocampus, the entire S-shaped hippocampal cortex bends back on itself, and each cornu Ammonis (CA) field becomes much more difficult to differentiate with precision. Similarly, at caudal levels the simple S-shape is obscured as the formation bends near the splenium of the corpus callosum. The hippocampus continues as a small band of neurons (the *indusium griseum*) above the entire length of the corpus callosum; this band of neurons extends around the genu of the corpus callosum and descends into the septal area, where it is known as the *taenia tecta*.

The hippocampus is one of the most easily discriminated regions within the cerebral cortex in Nissl-stained material (Fig. 4), and in magnetic resonance imaging (MRI) scans it is also clearly visible (Fig. 3). Most of the hippocampus has a single compact layer of neuronal cell bodies and thus contrasts sharply with the five neuronal cell layers present in most areas of neocortex (Fig. 4). This relative simplicity has led many researchers to use the hippocampus as a model system to illuminate the structure and function of the more complex neocortex.

Although some differences in nomenclature continue to confuse the hippocampal literature, the following terms are most widely used and are best defined. The *hippocampal formation* consists of two facing and overlapping horseshoe-shaped cell layers, the *dentate gyrus* and the *hippocampus proper* (Ammon’s horn, or cornu Ammonis [CA]; Fig. 5). However, in most common usage, and in this chapter,

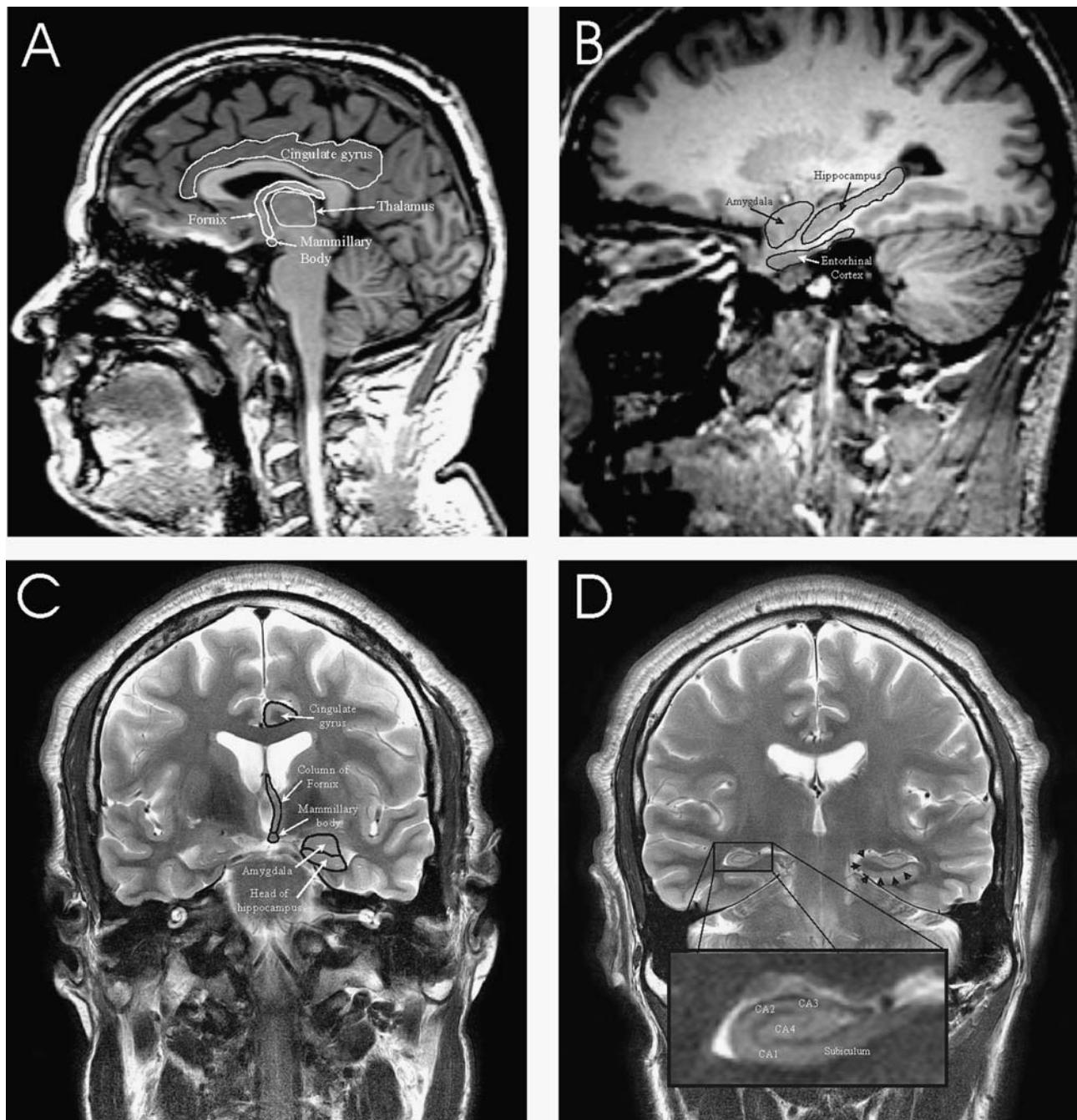


Fig. 3. (A) and (B) are MRI scans of sagittal sections and (C) and (D) are coronal MRI scans through the human brain illustrating the location of the limbic system; black arrowheads in (D) indicate the entorhinal cortex.

the term *hippocampus* is used instead of *hippocampal formation*. The hippocampus proper is divided into three CA regions: CA1, CA2, and CA3 (adapted from Lorente de Nó; see Fig. 4). Rose (1938) divided the hippocampus proper into five fields, designated h1 through h5, and his terminology remains popular among some neuropathologists. The *subiculum*, which is an extension of the CA region, is sometimes

considered to be a part of the hippocampus, but usually it is seen as a separate entity.

2.1.2. REGIONS OF THE HIPPOCAMPUS

The *dentate gyrus* is the cell layer that caps the distal tip of the CA fields (Fig. 4 and Fig. 6). The main cell layer is composed of tightly packed granule cell neurons that have apical dendrites that extend

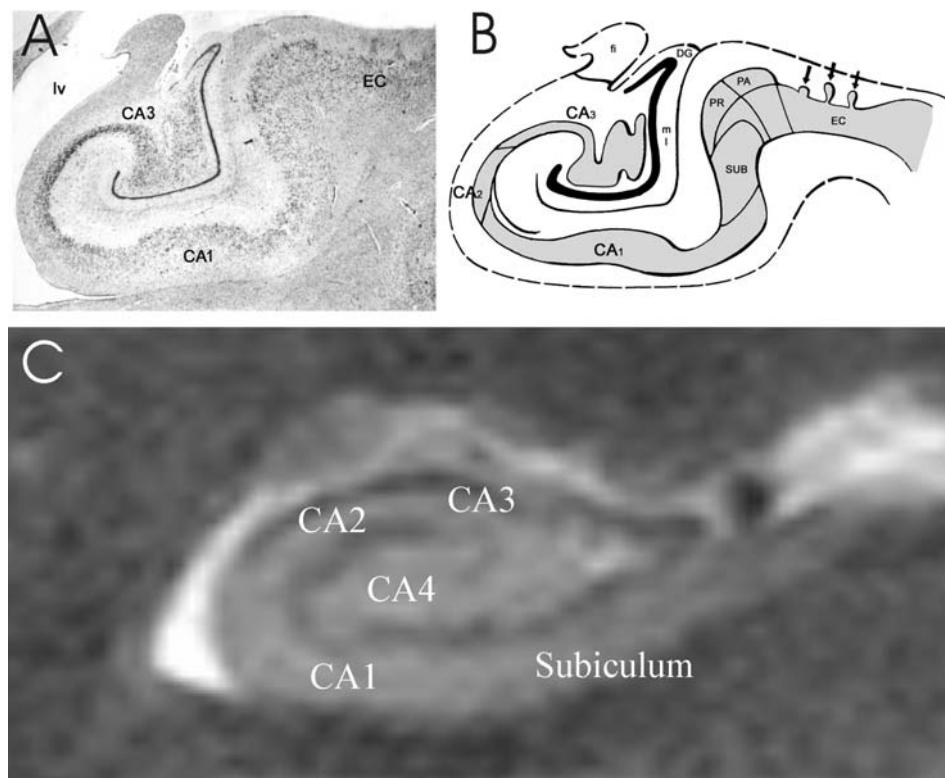


Fig. 4. (A) Nissl-stained coronal section of a human hippocampal formation taken from the mid-anteroposterior level. (B) Line drawing that illustrates the divisions of the hippocampal formation. (C) High-power MRI scan of the hippocampus. CA, the fields of the hippocampus proper; DG, dentate gyrus granule cell layer; EC, entorhinal cortex; fi, fimbria; lv, lateral ventricle; ml, dentate gyrus molecular layer; PA, parasubiculum; PR, presubiculum; SUB, subiculum.

toward the pial surface (Fig. 6, A2), but the granule cell neurons also give rise to less conspicuous basal dendrites in the hilus. These granule cells are generated rather late in embryonic development, and some of these neurons continue to divide into adulthood. Although generation of new neurons was known to occur in the dentate gyrus, more recent work demonstrates that neurons also proliferate in a few other places (primarily in the *rostral migratory stream*) in the mature brain.

Superficial to the granule cell layer lies the molecular layer, which contains most of the dendrites of the granule cells, and deep to the granule cell layer lies the polymorphic layer or *hilus*. The axons of the polymorphic neurons in the hilus project to the molecular layer of the dentate gyrus.

The hippocampus proper consists of one pyramidal cell layer that is several neurons thick. On the basis of cellular morphology, the hippocampus proper has been divided into an area that lies proximal to the dentate gyrus (i.e., area CA3); an area that lies distal to the dentate gyrus (i.e., area CA1), which contains neurons that are slightly smaller in size and

more widely scattered; and a short cell region, area CA2, which lies between CA3 and CA1 (Fig. 4 and Fig. 6).

Area CA3 has a relatively compact pyramidal cell layer; within the dentate hilar area, CA3 bends toward one blade of the dentate gyrus and then bends back toward the opposite blade (Fig. 4). In addition to the relatively large size of the CA3 pyramidal neurons, the structure of the apical dendrites differentiates these neurons from those in CA2 and CA1. The apical dendrites of the CA3 pyramidal neurons bifurcate close to their soma, but the apical dendrites of the CA1 and CA2 pyramidal neurons have smaller branches along most of their length (Fig. 6, A1).

The granule cell neurons of the dentate gyrus send their axons, the *mossy fibers*; they are so-called because of the large terminals that are formed by these axons on the CA3 neurons. These axons terminate massively on the proximal shaft of the apical dendrites (Fig. 6B). Above the molecular layer of the CA fields is the obliterated *hippocampal fissure*. The layer where the basal dendrites of the pyramidal

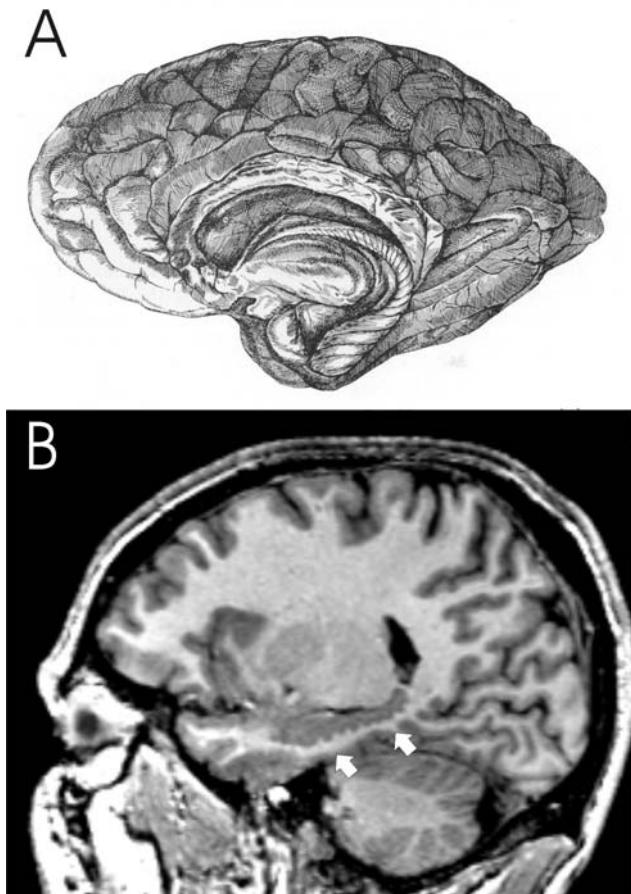


Fig. 5. (A) The 18th century French physician Duvernoy demonstrates, with some imagination, why the hippocampus resembles Ammon's horn (*cornu Ammonis*; shown here as a slightly modified drawing). (B) Sagittal MRI scan of human brain showing the hippocampus as Ammon's horn; the amount of hippocampal dentation varies extensively between humans (arrows indicate hippocampus).

cells reside is called *stratum oriens*, where scattered basket and polymorphic neurons are also located. Between *stratum oriens* and the lateral ventricle lies the *alveus*, a major fiber bundle that carries information to and from the hippocampus. Many axons in the *alveus* course through the *fimbria*, a large fiber bundle that lies medial and dorsal to the CA fields (Fig. 4), and *fornix* en route to subcortical and diencephalic sites (Fig. 3).

The borders of area CA2 are not easily discriminated because some neurons of CA3 tend to lie deep to the CA2 pyramidal cells. CA2 contains many large pyramidal neurons that are similar in size to those in CA3, but CA2 has a more compact pyramidal cell layer. In contrast, CA1 has slightly smaller and more widespread pyramidal neurons. Further, the mossy fibers do not extend to the CA2 neurons (Fig. 6), and

the CA2 neurons can be selectively labeled for some neurotransmitters.

2.2. The Subiculum Cortices Serve as the Output Area of the Hippocampus

The human subiculum cortices have four major divisions (i.e., prosubiculum, subiculum, parasubiculum, and presubiculum). The *subiculum proper* has a much wider neuronal cell layer than does CA1 and can be subdivided into a superficial (large cell) and deep (small cell) sublayer. Researchers have shown that there is a prosubicular division between CA1 and subiculum proper, but the differences between this area and the subiculum are not easily distinguishable. The border of subiculum with *presubiculum* (area 27 of Brodmann) is marked by the appearance of a tightly packed layer of small pyramidal neurons that caps the inner layer that appears in the same position as the subiculum's pyramidal cell layer. As in all areas of the hippocampal formation, adjacent areas tend to slide over or under each other in transition regions (Fig. 4). The parasubiculum (i.e., area 48 of Brodmann) lies between the presubiculum and the entorhinal cortex. Similar to the presubiculum, the parasubiculum has two major neuronal cell layers, but the neurons in the outer layer are larger than those in the corresponding layer of the presubiculum.

2.3. The Parahippocampal Region Is the Area Responsible for Providing Input to the Hippocampus and Subiculum

In humans, the parahippocampal region consists of the following three cortical areas, entorhinal, perirhinal, and parahippocampal cortices. The human entorhinal cortex (area 28 of Brodmann) lies ventral to the rostral half of the hippocampal formation and the amygdaloid complex (Fig. 3). The entorhinal cortex has been divided into a number of subfields, from 5 to as many as 23 separate areas; modern studies use a subdivision into 8 subfields, but the cortical area is only subdivided in its 2 major subdivisions (medial and lateral areas) in this chapter. The differentiating elements of the entorhinal cortex are present in layers II and IV. The neuronal cell bodies in layer II form large, prominent cell islands, especially at the rostral levels of the entorhinal cortex. In contrast, layer IV consists of a dense fiber plexus (i.e., the lamina dissecans) that clearly separates the small pyramidal

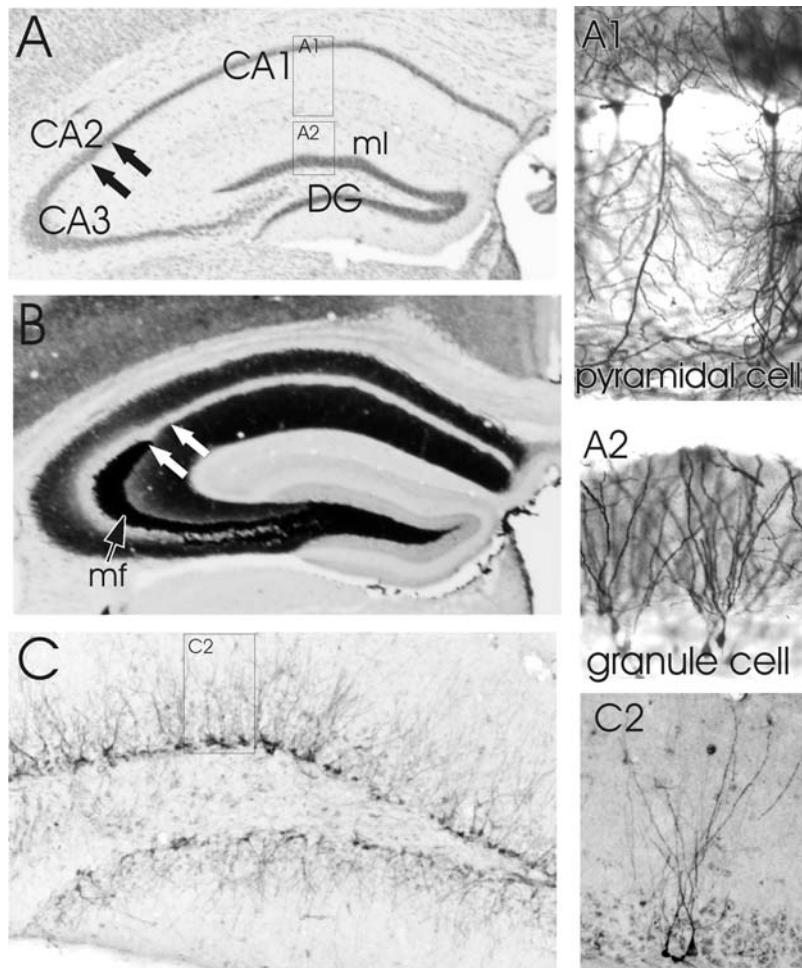


Fig. 6. (A) Nissl-stained coronal section of a mouse hippocampus; A1 and A2 show the Golgi-stained appearance of pyramidal neurons and granule cells in the CA and dentate gyrus fields, respectively. (B) The adjacent, Timms-stained section (indicating the density of metals in tissue), which illustrates the divisions of the hippocampal formation; the arrow indicates the mossy fibers. (C) The dentate gyrus of the mouse stained with Doublecortin, a marker for neural stem cells; C1 shows two of these “granule” cells at higher magnification. Arrows indicate border of CA2 area, CA, the fields of the hippocampus proper; DG, dentate gyrus granule cell layer; ml, dentate gyrus molecular layer.

neurons in layer III from the larger pyramidal neurons in layer V. The entorhinal cortex is laterally bordered by areas 35 and 36 (i.e., the perirhinal cortex). Although the entorhinal cortex was named for its proximity to the rhinal sulcus in subprimates and monkeys, the rhinal sulcus in humans is rostral to the entorhinal cortex. In humans, the collateral sulcus forms the border of the parahippocampal gyrus, but it does not faithfully mark the lateral border of the entorhinal cortex, which lies in the medial part of the parahippocampal gyrus. The other areas of the parahippocampal region (postrhinal cortex and areas TH and TF) provide major inputs (primarily visual) to the perirhinal and entorhinal cortices.

2.4. The Septal Nuclei Are Reciprocally Connected with the Hippocampus

The *septal complex* is a subcortical telencephalic region that is interconnected with the hippocampus through the fornix, and it lies near the genu of the corpus callosum and medial to the lateral ventricles. Few or no neuronal cell bodies are within the septum pellucidum, the thin sheet of tissue that is dorsal and caudal to the anterior commissure and separates the lateral ventricles. Most septal neurons lie in the septal nuclei, which are divided into two major divisions, the *medial* and *lateral septal nucleus*. The lateral septal nucleus receives input from the hippocampal formation; in contrast, the medial septal nucleus innervates

(with cholinergic or GABAergic axons) the hippocampal formation (and entorhinal cortex). In addition to the medial and lateral nuclei, the septal complex also includes the *septohippocampal*, *septo-fimbrial*, and *triangular septal nuclei*, the *bed nucleus of the stria terminalis*, and the *diagonal band of Broca*. The septal nuclei are densely interconnected with the rostral part of the hypothalamus (primarily the preoptic and anterior hypothalamic areas).

2.5. The Mammillary Complex Is a Limbic Hypothalamic Area

Although several areas of the hypothalamus are interconnected with limbic cortical regions, the mammillary bodies have the most prominent connections with the limbic system. The *medial mammillary nuclei* bulge out from the base of the hypothalamus, thus giving rise to their suggestive name (Fig. 3). The mammillary complex is typically divided into four nuclei. The largest nucleus is the *medial mammillary nucleus*, lateral to which is the *lateral mammillary nucleus*, and further lateral and dorsal is the *tubero-mammillary nucleus*. Finally, above the medial mammillary nucleus is the *supramammillary nucleus*. One main input to the mammillary bodies is through the postcommissural fornix and derives from the subiculum cortices, the other major input arises from the ventral tegmental nucleus, but they also receive input from the hypothalamus and septal areas. The main output from the mammillary bodies is through the *mammillothalamic tract*, which terminates in the anterior thalamic nuclei.

2.6. The Anterior Thalamus Is the Primary Limbic Thalamic Area

The anterior nuclei of the thalamus are situated dorsomedial to the internal medullary lamina and classically are divided into three nuclei: the *anterior dorsal*, *anterior medial*, and *anterior ventral thalamic nuclei*. In lower primate and subprimate species, the three nuclei are quite distinct, but in the human brain, the anterior medial nucleus is difficult to discriminate from the anterior ventral nucleus, and the two have often been grouped together and called the nucleus anterior principalis, but the connections of these areas are very different. The anterior dorsal nucleus is by far the smallest of the three; in all species, the cytoarchitecture of the anterior dorsal nucleus is distinct from that of the anterior ventral and the anterior medial nuclei, because its neurons are more tightly packed, and they stain darker than in the other thalamic nuclei.

The *lateral dorsal nucleus* lies on the dorsal surface of the thalamus, and although its Nissl-stained appearance and location are distinct from those of the anterior nuclei proper, many of its connection are similar, therefore it is usually considered as part of the anterior thalamic group.

Several other thalamic nuclei have major projections to the limbic cortex. These include the *medial dorsal nucleus*, the *midline nuclei* (especially the parataenial, the paraventricular, and the reunions nuclei), the *intralaminar nuclei*, and the *lateral thalamic nuclei* (i.e., lateral posterior and medial pulvinar nuclei). Modern tract tracing studies have shown that only parts of these nuclei can be considered limbic and that other regions of these nuclei are primarily related to nonlimbic areas of neocortex.

2.7. The Cingulate Cortex Comprises the Dorsal Limbic Midline Cortex

The major projection from the anterior thalamic nuclei is to the cingulate cortex, an area that forms a major component of the circuit of Papez (Fig. 2). This cortical region lies below the cingulate sulcus and caps the corpus callosum from its rostrum to its splenium (Fig. 1 and Fig. 3). A major division of the cingulate cortex lies immediately behind the splenium of the corpus callosum, deep within the sulcus of the corpus callosum. This part of the cingulate cortex, which is also referred to as retrosplenial cortex, includes areas 26, 29, and 30 of Brodmann; the part of cingulate cortex that is rostral to the splenium of the corpus callosum includes areas 23, 24, 25, 32, and 33.

2.8. The Amygdala Is Divided into Three Major Regions

The amygdala, whose name reflects its almond-like appearance, is a mass of gray matter that is situated within the temporal lobe, immediately rostral to the inferior horn of the lateral ventricle. Early anatomists thought that the amygdala was part of the basal ganglia, but later connectional, developmental, and immunohistochemical studies have demonstrated that it is connected with and functions as part of the limbic system. Modern studies see the amygdala as the core of the extended amygdala concept; in this model, the amygdala extends further rostral into the basal forebrain. The extended amygdala contains, next to the medial and central nuclei of the amygdala, the ventral pallidum, parts of the nucleus accumbens, and most of the bed nucleus of the stria terminalis.

Nowadays, the amygdala is usually divided into three major regions: the *cortical* (or superficial) nuclei, the *basolateral* (or deep) nuclei, and the *centromedial* nuclei. The basolateral nuclei consist of four nuclei: the lateral, the basal lateral, and the basal medial nuclei and the amygdaloclastral area. The cortical group of nuclei includes the anterior and the cortical amygdaloid nuclei, the periamygdaloid cortex, and the nucleus of the lateral olfactory tract, a nucleus that is poorly developed in human brain. The centromedial nuclei include the central and the medial nuclei. On the basis of its structure and connections, the central nucleus is considered as a separate region from the two large nuclear groups of the amygdala. At its caudal end, the superficial group of nuclei is continuous with the amygdalohippocampal area, a transition zone between the amygdala and the hippocampal formation.

3. MAJOR CONNECTIONS OF THE LIMBIC SYSTEM

3.1. *The Entorhinal and Subiculum Cortices Are the Primary Gateways to and from the Hippocampus*

3.1.1. AFFERENT CONNECTIONS OF THE HIPPOCAMPUS

The majority of extrinsic inputs to the hippocampus originate in the *entorhinal cortex* or *septal nuclei*. The entorhinal cortex receives inputs from several areas of the temporal, frontal, and midline cortices. The perirhinal cortex and the temporal polar cortex project to the lateral portion of the entorhinal cortex, whereas the dorsal temporal, insular, orbitofrontal, infralimbic, prelimbic, and cingulate cortices have significant projections to medial parts of the entorhinal cortex. Several subcortical areas also have significant direct projections to the entorhinal cortex. The lateral nucleus of the amygdala has a dense projection to the lateral entorhinal cortex, the claustrum also projects to the entorhinal cortex, as do the paraventricular and reunions nuclei of the thalamus.

The cortical areas that project to the entorhinal cortex have widespread inputs from almost all associational cortices. Thus, the inputs to the entorhinal cortex provide sensory and motor information to the dentate gyrus and hippocampus, but a few other extrinsic inputs directly project onto the dentate granule cells and the CA pyramidal neurons. The most prominent of these projections originates in the

medial septal nucleus and nucleus of the diagonal band of Broca and reaches the hippocampus through the fornix. Approximately 50% of the septal axons in this projection use acetylcholine as a neurotransmitter, whereas the other half uses GABA. A second direct projection to the dentate gyrus and CA fields originates in the supramammillary nucleus, and the nucleus reunions of the thalamus has a prominent projection to the CA1 field. These subcortical inputs are generally not seen as carrying information, but they help in setting the tone of processing in the hippocampus, such as stress or emotional level.

Several brain-stem nuclei that project to most of the telencephalon also project to the hippocampal formation. These include a noradrenergic projection from the *locus coeruleus*, a serotonergic projection from the *raphe nuclei* (i.e., dorsal raphe and central superior nuclei), and a smaller, dopaminergic projection from the *ventral tegmental area of Tsai*. The *periaqueductal gray*, the lateral and dorsal tegmental nuclei, and reticular nuclei of the brain stem also project to the hippocampal formation.

3.1.2. INTRINSIC CONNECTIONS OF THE HIPPOCAMPUS

The main intrinsic connections of the hippocampal formation form a serial pathway, but several collateral and feedback projections are added onto the serial path. The serial connection path of the hippocampal formation is traditionally called the *trisynaptic pathway* (i.e., the sequential projections from the entorhinal cortex to the dentate gyrus to CA3 and then to CA1) (Fig. 7). The entorhinal cortex projects to the dentate gyrus granule cells but has collaterals to the CA fields and the subiculum as well. The largest input to the dentate granule neurons originates in layer II neurons of the entorhinal cortex; the axons terminate in the molecular layer of the dentate gyrus on the outer two-thirds of the granule cell dendrites. In contrast, the input to the CA fields originates in the layer III neurons of the entorhinal cortex. Another connectivity map exists in these connections (i.e., the position of the cells of origin within the entorhinal cortex dictates the position of the terminals along the septotemporal axis of the hippocampus). In monkey, axons of neurons in lateral parts of the entorhinal cortex terminate in the septal (dorsal) part of the hippocampus, whereas axons originating in medial parts of the entorhinal cortex terminate in the temporal part of the hippocampus.

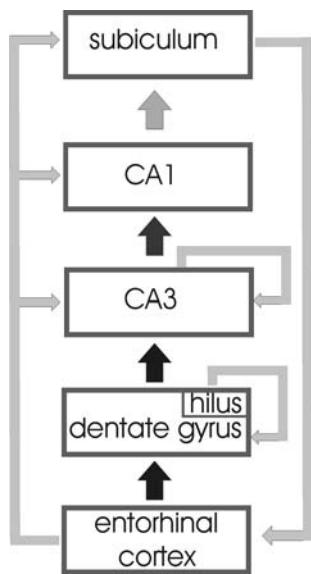


Fig. 7. The *black arrows* designate the trisynaptic pathway of the hippocampus, and the feedback and feedforward collaterals are shown by the *gray arrows*.

The granule cells of the dentate gyrus project to the pyramidal neurons in area CA3 through their mossy fibers (Fig. 6). The CA3 pyramidal neurons send their axons, known as the *Schaffer collaterals*, to fields CA2 and CA1, in turn CA1 has a massive projection to the subiculum. The subiculum, among its many outputs, also projects back to the entorhinal cortex (Fig. 7). The other two areas of the subicular cortices (i.e., the presubiculum and the parasubiculum) do not receive direct input from the hippocampus, but they give rise to dense projections to the entorhinal cortex, with presubiculum axons terminating in layers I and III and parasubiculum axons terminating in layer II of the entorhinal cortex.

Several feedback loops and local projections of interneurons modify the information flow in the hippocampal formation (Fig. 7). The polymorphic neurons of the dentate hilus project back to the proximal dendrites and cell bodies of the granule cells and potently influence information flow through the granule cells. Similarly, the interneurons in the CA fields provide local feedback to the CA pyramidal neurons. For instance, the primary cell layer of all CA fields and of the dentate gyrus contains basket cell neurons that potently inhibit neurons in the layer in which they reside. In contrast with all other hippocampal areas, the CA3 neurons have extensive intrinsic connections (i.e., *excitatory*) within the CA3 field, which is likely the cause of its susceptibility to develop epileptic foci.

3.1.3. EXTRINSIC CONNECTIONS OF THE HIPPOCAMPUS

Although CA3 and CA1 neurons give rise to significant projections to the septal nuclei, and CA1 has significant projections to the prefrontal cortex and entorhinal cortex, most of the major outputs of the hippocampal formation arise from the subiculum. The *postcommissural fornix* originates in the subicular cortices; these axons innervate the anterior and lateral dorsal thalamic nuclei, the ventromedial hypothalamic nucleus, and the mammillary complex. The densest of these projections ends in the mammillary complex. The prosubiculum also has a substantial amygdaloid projection that terminates in the basal nucleus as well as in the periamygdaloid nucleus.

The subiculum projects to the cingulate, retrosplenial, and medial orbital cortices and to the parahippocampal gyrus, including the entorhinal and perirhinal cortices (Fig. 8). The entorhinal cortex provides a dense projection to the cerebral cortex, where its axons terminate prominently in perirhinal, caudal parahippocampal, retrosplenial, and temporal polar cortices. The entorhinal cortex has a small projection to the lateral and basal nuclei of the amygdala. In turn, the cortical areas that are innervated by the subiculum and entorhinal cortices project have widespread connections with almost all associational cortices.

3.2. AXONS TO AND FROM THE SEPTAL NUCLEI COURSE IN THE FORNIX AND THE MEDIAL FOREBRAIN BUNDLE

The septal nuclei receive a major input from the hippocampus and project densely to the hippocampus; these axons course through the fornix. The septal

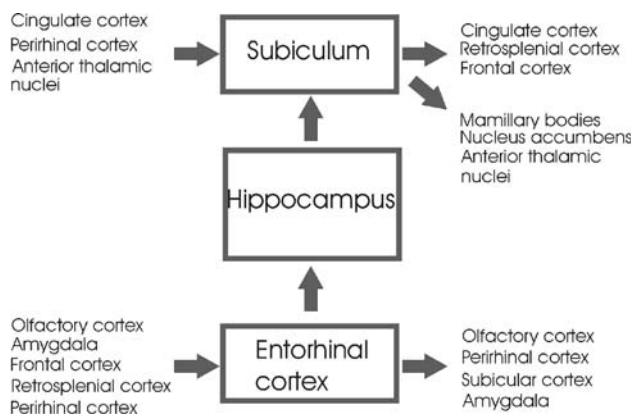


Fig. 8. Most of the input to the hippocampus arises in the entorhinal cortex, and most of its output flows through the subiculum. The primary extrinsic connections of the subiculum and entorhinal cortex are illustrated.

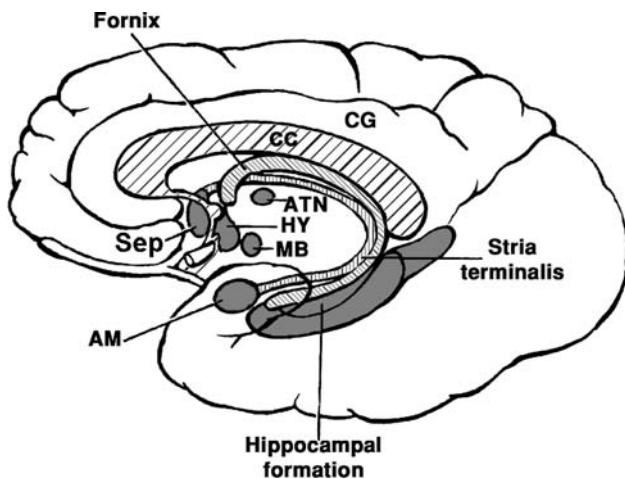


Fig. 9. This figure depicts the major fiber bundles related to the limbic system. The stria terminalis carries the primary output axons from the amygdala (AM); the ventral amygdalofugal pathway is not illustrated. The other major fiber bundle, the fornix, connects the hippocampal formation to subcortical and diencephalic sites. ATN, anterior thalamic nuclei; cc, corpus callosum; CG, cingulate gyrus; HY, hypothalamus; MB, mammillary bodies; NBF, basal forebrain including nuclei accumbens, diagonal band of Broca, and nucleus basalis; Sep, septal nuclei.

nuclei are also innervated by the amygdala, the hypothalamus, and the medial midbrain reticular region through the medial forebrain bundle. Efferent axons of the septal nuclei course through the *stria medullaris* and the *medial forebrain bundle* to terminate in the habenula and hypothalamus, respectively (Fig. 9). A few of the medial forebrain bundle axons from the septal nuclei extend to midbrain tegmentum and other brain-stem nuclei.

3.3. The Mammillary Bodies Relay Hippocampal Information to the Anterior Thalamic Nuclei

One main input to the mammillary bodies arises from the subicular cortices and runs through the *fornix*. The *fornix* splits into two segments at the anterior commissure, and the postcommissural complement of axons traverses the hypothalamus and terminates in the mammillary complex. Another main input to the mammillary complex arises in the dorsal and ventral tegmental nuclei, and the anterior hypothalamus also innervates the mammillary complex.

The majority of efferent axons leave the mammillary nuclei by way of the *mammillothalamic tract* (ascending) and the *mammillotegmental tract* (descending). Through the *mammillothalamic tract*, the

medial mammillary nucleus projects to the anterior ventral and anterior medial thalamic nuclei, whereas the lateral mammillary nucleus projects primarily to the anterior dorsal nucleus. The *mammillotegmental tract* carries axons to the dorsal and ventral tegmental nuclei in the midbrain. The supramammillary nuclei have a small but significant direct projection to the dentate gyrus.

3.4. The Anterior Thalamic Nuclei Project Primarily to the Posterior Cingulate Cortex and to the Subicular Cortices

The anterior thalamic nuclei are innervated by three major areas. The *mammillothalamic tract* and *corticthalamic fibers* from the cingulate and the retrosplenial cortices supply approximately equal numbers of axons to all nuclei. Axons arising from the subicular cortices provide the third major afferent. The lateral dorsal nucleus also receives prominent inputs from retrosplenial and subicular cortices but only a small input from the mammillary complex.

The thalamocortical projections from the anterior thalamic nuclei innervate the entire cingulate, retrosplenial, and subicular cortices, however each of the thalamic nuclei ends in distinct regional and laminar patterns (Fig. 10). Unlike most thalamic projections, which terminate primarily in layer IV of the cortex, the projections from the anterior and lateral dorsal nuclei primarily terminate in both layers I and IV of the cortex. The anterior medial nucleus also projects to the prefrontal and orbitofrontal cortices.

The lateral and medial thalamic nuclear groups also project to the limbic cortex. The projection from the lateral dorsal nucleus largely overlaps the projections of anterior dorsal and anterior ventral

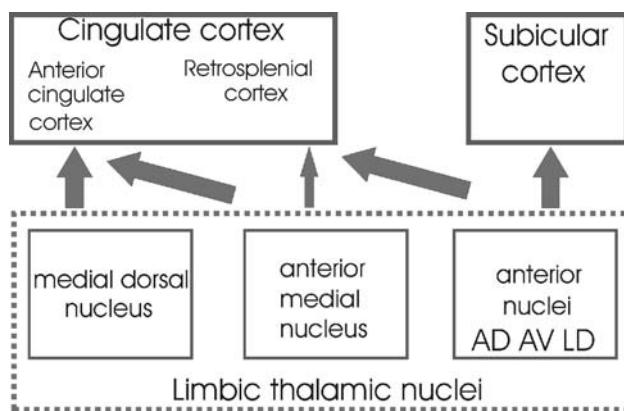


Fig. 10. Each of the limbic thalamic nuclei that project to the cingulate cortex and hippocampus has a distinct terminal field.

thalamic nuclei to the posterior limbic cortex and extends into the medial parietal cortex. The medial pulvinar thalamic nucleus has projections to the posterior cingulate and retrosplenial cortex in primates. Parts of the medial dorsal nucleus project to the rostral cingulate cortex (i.e., Brodmann areas 25 and 32), and the medial subnucleus of the ventral anterior thalamic nucleus has projections to the cingulate cortex.

3.5. In Contrast with the Anterior Cingulate Cortex, the Posterior Cingulate Cortex Is Connected to the Hippocampal Formation

3.5.1. AFFERENT CONNECTIONS

The thalamic projections to the cingulate cortex are widespread, but the cortical connections are equally diverse. The entire cingulate cortex, including the retrosplenial cortex, is interconnected by extensive commissural and associational projections, with the latter linking up the posterior and anterior segments. Much of the caudal cingulate cortex also receives extensive projections from the subiculum, and from several neocortical areas, including somatosensory, prefrontal, and association cortices (e.g., visual, parietal, and auditory cortices).

Similarly to other parts of the cortex, the cingulate cortex is innervated by cholinergic (i.e., from the diagonal band of Broca), noradrenergic (i.e., from locus coeruleus), and serotonergic (i.e., from the dorsal raphe and central superior nuclei) axons. The ventral tegmental area of Tsai has a dense dopaminergic projection to the anterior cingulate cortex (but it has only a sparse projection to the posterior cingulate cortices).

3.5.2. EFFERENT CONNECTIONS

The projections of the posterior cingulate cortices to the subicular and entorhinal cortices close the hypothesized circuit of Papez (Fig. 2). This projection is relatively dense but arises primarily in the retrosplenial cortex. The cingulate cortex projects to many areas of associational neocortex, including somatosensory, prefrontal, and association cortices. Further, all areas of the cingulate cortex project to the medial striatum, and all areas are connected to the anterior (and midline) thalamic nuclei.

The anterior cingulate cortex also has substantial projections to the hypothalamus and brain stem, especially to areas related to visceral regulation. The hypothalamic projection arises primarily from Brodmann areas 23, 24, 25, and 32 and terminates primarily in the lateral hypothalamic area. Areas 24 and 25

of the anterior cingulate cortex also innervate the midbrain, and these axons terminate in the periaqueductal gray matter, the raphe nuclei, and the deep layers of the superior colliculus.

3.6. The Amygdala Is Connected to Both the Cerebral Cortex and the Hypothalamus

3.6.1. AFFERENT CONNECTIONS

The major afferent connections of the amygdala differentiate the three components of the complex (Fig. 11). The *olfactory bulb* projects directly to the corticomедial amygdaloid nuclei through the lateral olfactory tract; the olfactory bulb and anterior olfactory nucleus projections terminate in the anterior cortical nucleus, the nucleus of the olfactory tract, and the periamygdaloid cortex. Areas of the basolateral nuclear group receive an olfactory-related input, but this arises in the piriform cortex and from intrinsic amygdaloid projections from the superficial nuclear group. The only major area of the amygdala that lacks either an olfactory bulb or a direct olfactory cortex input is the *central nucleus*.

The amygdala, especially the lateral nucleus, is innervated directly by several other unimodal sensory areas of the cerebral cortex; in addition to these single modality sensory inputs, most other parts of the temporal lobe supply polysensory inputs to the amygdala. Most of these projections terminate in the lateral and basal nuclei. Somatosensory information reaches the amygdala through projections from the *insular and orbital cortices*. The most direct pathway for this information is from the secondary somatosensory cortex to the posterior insular cortex, which projects heavily to the lateral nucleus of the amygdala. The caudal orbital cortex and the anterior cingulate cortex (areas 24, 25, and 32) also have projections to the basal nucleus.

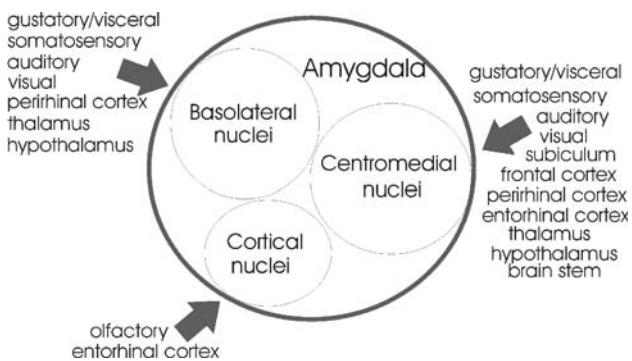


Fig. 11. The primary cortical inputs to the amygdala are shown.

Several *thalamic nuclei* project to the amygdala. Auditory input is directed to the lateral and central nuclei through projections from the medial geniculate nucleus. Gustatory projections reach the lateral nucleus from the ventral posterior medial nucleus (i.e., pars medialis). Other thalamic projections to the amygdala originate in the midline, intralaminar, and the medial pulvinar nuclei.

The *hypothalamus* sends a moderately dense projection to the amygdala, which reciprocates the amygdalohypothalamic projections. The most prominent projections originate in the ventromedial and lateral hypothalamic nuclei and terminate in the basolateral nuclei of the amygdala.

A significant cholinergic projection to the amygdala originates from the magnocellular neurons of the basal forebrain. Specifically, the nucleus basalis of Meynert and the horizontal and vertical limbs of the diagonal band of Broca project to the basolateral and cortical nuclei, and the substantia innominata projects to the central amygdaloid nucleus. Brain-stem projections to the amygdala arise in the parabrachial nucleus (to the central nucleus), the pedunculopontine nucleus, the ventral tegmental area of Tsai (i.e., dopaminergic), and the locus coeruleus (i.e., noradrenergic).

3.6.2. INTRINSIC CONNECTIONS

The intrinsic connections of the amygdala have been difficult to resolve with precision because of the small, irregular size of many amygdaloid nuclei and the fact that many axons pass through nuclei in which they do not terminate. With the advent of modern tracing techniques that minimize these problems, the intrinsic circuitry of the amygdala has begun grudgingly to give way. The lateral nucleus, which receives the most direct sensory inputs, projects to all subdivisions of the amygdala. The basal nuclei project to all other amygdaloid nuclei except the lateral amygdaloid nucleus. The central and superficial nuclei have many intra-amygadaloid connections, but few of these projections are to the basolateral nuclei. These connections suggest that processing through the amygdala is primarily in series.

3.6.3. EFFERENT PROJECTIONS

The amygdala has axonal projections to many cortical areas; both the entorhinal cortex and the subiculum cortex receive a substantial projection from the amygdala. The basal amygdaloid nuclei project densely to several areas of the unimodal sensory cortex that project primarily to the lateral nucleus of the amygdala (Fig. 12). The amygdala also projects to

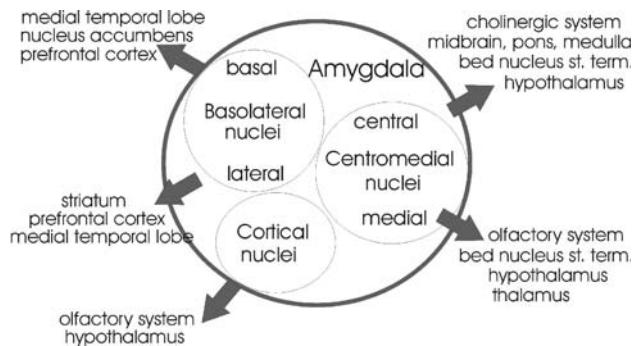


Fig. 12. The primary cortical outputs from the amygdala are shown.

many polysensory regions of the cortex, and many of these projections arise from the lateral nucleus. Within the temporal lobe, the rostral part of the temporal cortex receives the densest amygdaloid projections, but most of the temporal lobe and much of the occipital lobe are innervated to some extent. The amygdala also projects densely to the anterior cingulate cortex (i.e., areas 23, 24, 25, and 32) and the frontal and insular cortices.

Two major fiber tracts connect the amygdala with the diencephalon and brain stem (Fig. 9). The *stria terminalis* is the more obvious of the two, as it arches along medial to the entire extent of the body and tail of the caudate nucleus and descends lateral to the fornix at the level of the anterior commissure. Most axons in the stria terminalis terminate in the bed nucleus of the stria terminalis, which is located dorsal to the anterior commissure. This nucleus, which is considered by some to be a rostral extension of the amygdala, has dense projections to the hypothalamus and brain stem. Amygdalar axons in the postcommissural stria terminalis project to the anterior hypothalamus, and some of these axons course further caudally to the brain stem through the medial forebrain bundle. Other amygdalar fibers terminate in the preoptic region and the ventromedial hypothalamic nucleus.

The *ventral amygdalofugal tract* provides a second pathway for amygdaloid axons that are destined for subcortical, diencephalic, and brain-stem areas. This projection passes rostral from the amygdala, courses beneath the lenticular nucleus through the substantia innominata, and terminates in the lateral preoptic nucleus and the lateral hypothalamic area. Caudal to the hypothalamus, axons from the central nucleus of the amygdala terminate in several brain-stem nuclei. Amygdalofugal fibers also terminate in the medial dorsal thalamic nucleus; these axons originate

from many areas of the amygdala (the medial and the central nuclei are exceptions) but especially from the basolateral nuclei and the periamygdaloid cortex. There is also a reciprocal connection to the midline thalamic nuclei (i.e., the paraventricular and paratenial nuclei), primarily from the central and medial amygdaloid nuclei. The central and basal amygdaloid nuclei have a reciprocal connection with the basal forebrain. One of the densest amygdalofugal projections is that from the basal nuclei to the striatum, and much of this terminates in the “limbic” striatum (i.e., the nucleus accumbens), but there is also a substantial projection to the ventromedial caudate and putamen.

4. FUNCTIONAL CONSIDERATIONS

4.1. *The Limbic System, Especially the Hippocampus, Contributes to Normal Learning and Memory*

Before discussing the relationship between the limbic system and learning and memory, it is useful to clarify certain terms that define memory. Memory can be divided into *short-term memory*, which endures for a very brief time, and *long-term memory*, which is retained long after the event. Long-term memories are further divided into *declarative memory*, which refers to the memory of facts that can be recalled to consciousness, and *procedural memory*, which refers to patterns of behavior that are learned (e.g., motor skills, procedural skills). The limbic system appears to be primarily involved in the transfer of declarative memory from short-term to long-term memory.

Much of our early understanding of the relation between learning and memory and the limbic system came from studies of patients with damage to the brain. Deficits in many patients have confirmed the role of the posterior limbic cortex in mnemonic tasks, but patient R.B. provides the clearest clinical picture of the relation because rigorous testing of this patient has been followed by clear histologic findings. While undergoing coronary bypass surgery, R.B. had a transient ischemic episode, after which he displayed severe anterograde amnesia (recalling new memories) but no significant retrograde amnesia (recalling old memories). Postmortem examination 5 years after the ischemic episode revealed a very selective bilateral lesion of the CA1 field of the hippocampus, and no apparent damage to any other limbic region of the brain. Remarkably, the severe deterioration of declarative memory ability in R.B. was not accompanied by any apparent alteration in emotion or cognitive function.

Animal studies have supported the role of the hippocampus and posterior limbic cortex in mnemonic tasks. In monkeys, damage to the hippocampal formation impairs memory but has little effect on emotional behavior. In contrast, selective damage to the amygdala causes affective alterations but few or no alterations in learning and memory.

Several other areas of limbic cortex also appear to contribute to learning and memory. As would be expected from the connections, damage to subiculum and entorhinal cortices affect learning and memory. The retrosplenial cortex, with which the hippocampal formation is highly interconnected, has been shown to participate in learning and memory.

4.2. *The Medial Temporal Lobe Memory System Is Part of the Limbic System*

The concept of the medial temporal lobe and its role in learning and memory functions has a long history. It was “discovered” in the late 1950s but it took many years before the relationship was widely accepted. In 1953, William Scoville operated on a patient (the now famous H.M.) who presented with intractable, severe generalized epileptic seizures. The bilateral temporal lobe resection was a successful treatment for the epileptic seizures, but it left the patient with a severe anterograde memory deficit (i.e., he was unable to convert short-term to long-term memory). The patient displayed very little retrograde memory deficit (i.e., he was able to recall events that occurred prior to his surgery). During the subsequent half century, this patient has been studied intensively. An example of H.M.’s dysfunctional state comes from the detailed 1968 report of Milner and colleagues, in which H.M. failed to recall almost any event that occurred after his surgery, even very traumatic events such as the death of his father. Only those events that were continuously repeated were remembered, and these were remembered only vaguely. In contrast with his obvious deficit in declarative memory, H.M. is quite accomplished in tasks requiring procedural learning skills, such as the mirror-draw task in which he shows normal memory. H.M., and many similar cases, demonstrate the importance of the temporal lobe in declarative memory, and they show that there is a dissociation between the areas of the brain that subserve declarative compared with procedural memory.

The medial temporal lobe plays a role in learning and memory; in humans it consists of both allocortical areas such as the amygdala, the hippocampus, the subiculum complex, and the entorhinal and perirhinal

cortices and neocortical areas such as the parahippocampal cortex. Today's prevailing view is that the medial temporal lobe subserves declarative memory but not perception, with the hippocampus contributing to remembering places and paths, whereas the perirhinal cortex "remembers" objects and the content of scenes. In this view, the amygdala does not contribute directly to memory or perception but it assigns biological significance (i.e., emotional content) to the memories.

4.3. The Limbic System Is Involved in Affective Behavior

Whereas learning and memory deficits appear to be the product of damage to more caudal limbic regions, especially the hippocampal formation, and the entorhinal and perirhinal cortices, the relation between emotional dysfunction and the limbic system appears primarily to involve the amygdala and anterior cingulate cortex.

A major insight into the neurologic mechanisms controlling affective state (i.e., personality, emotion, and social behavior) was Papez's 1937 paper in which he proposed that "limbic areas" of the brain were connected such that they formed a circuit that would play a role in coordinating emotion and emotional expression. Although this paper was speculative, it quickly gained considerable acceptance; in particular, Freudian psychoanalytic thinking readily welcomed the idea that the old and ancient parts of the brain were responsible for emotional and instinctive behavior and that phylogenetically newer areas of the brain, such as the neocortex, were primarily attentive to conscious tasks and controlled behavior.

In a study published in 1939, Klüver and Bucy reported that bilateral temporal lobectomy in monkeys produced dramatic behavioral changes, most of which could be defined as affective disorders. The *Klüver-Bucy syndrome* was characterized by markedly increased sexual activity that was often inappropriate (e.g., monkeys mounting other species or chairs), a loss of fear and a resulting flattening of emotions, an increase in oral behavior, and indiscriminate dietary behavior. Subsequent studies have demonstrated that it was the damage to the amygdala that caused the sexual, appetitive, and affective dysfunctions in these animals. The visual and memory losses that Klüver and Bucy reported in their original experiments have been shown to be the result of damage to other areas of the temporal cortex. The Klüver-Bucy syndrome occurs in humans who have

selective damage to the amygdala or have damage that includes the amygdala.

At around the time that Klüver and Bucy demonstrated the effects of amygdalectomy on emotional behavior, Jacobson and Fulton reported on their neurosurgical studies in chimpanzees and alluded to the calming effect that a prefrontal cortex lesion had on the behavior of one particularly neurotic female chimp. This led Egas Moniz, a prominent Portuguese neurologist, to return home immediately and begin treating a few patients with severe mental disorders using prefrontal lobotomy with positive effects. Moniz launched modern psychosurgery, for which he was awarded the 1949 Nobel Prize in Physiology and Medicine; the prize was also awarded for his role in the discovery of angiography.

During the 1940s through the 1970s, many clinicians carried out psychosurgery on various limbic regions, especially the prefrontal cortices, and for a time, these lesion techniques became the method of choice to relieve severe emotional disorders. In the United States, this idea of psychosurgery was energetically supported by the neurologist Walter Freeman, who developed the transorbital technique for frontal lobotomy. In the ensuing decades, criticism of psychosurgery increased until the method was abandoned in the 1970s, but only after tens of thousands of patients had been "treated." Except for the study of Klüver and Bucy and the work of a few other behaviorists, the method lacked an empirical basis.

Studies of patients who received these surgical interventions and the experimental literature in this field have demonstrated that the circuit of Papez is not a major contributor to emotional control. The area that Papez did not include in his circuit (i.e., the amygdala) or that he thought were peripheral contributors to the circuit (i.e., prefrontal cortex) have been shown to play a major role in emotional control and the conscious perception of emotional experience.

4.4. The Amygdala Is Involved in Fear and Emotions

The amygdala was part of MacLean's limbic system (i.e., the "visceral brain"). However, it did not stand out as an especially important limbic area until 1956 when Weiskrantz showed that the emotional components of the so-called Kluver and Bucy syndrome were due to the involvement of the amygdala. Weiskrantz proposed that amygdala lesions dissociate the affective or reinforcing properties of stimuli from their sensory representations. Thus, although the amygdala was known to be

involved in emotion for some time, much of the recent scientific interest in the amygdala stems from its role in fear. Research, mostly conducted in rats, has identified the amygdala as a central structure in the circuitry underlying fear conditioning. Recent studies in humans have complemented but also extended the basic findings from animals regarding the role of the amygdala in emotional processing. Emotion systems in the brain are generally viewed as belonging to the category of systems that form implicit memories. This does not imply that memories for emotional situations are only formed implicitly, as other systems, such as the explicit memory system of the medial temporal lobe, can form their own memories of emotional situations. It instead implies that the memories formed and stored by emotion systems are implicitly stored and accessed.

Studies on the neural systems of implicit emotional learning, emotion and memory, emotion's influence on attention and perception, social responding, and emotion inhibition and regulation indicate an important role for the amygdala. Although studies in humans cannot explore the neural systems of behavior with the same level of specificity as can research in nonhuman animals, identifying links in the neural representation of behavior across species results in a greater understanding of both the behavioral influence and neural representation of emotion in humans.

Recent studies suggest that fear stimuli automatically activate fear and capture attention. This effect is likely to be mediated by a subcortical brain network centered on the amygdala. Consistent with this view, brain imaging studies have shown that masked facial stimuli activate the amygdala as do masked pictures of threatening animals such as snakes and spiders. When the stimulus conditions allow conscious processing, the amygdala response to feared stimuli is enhanced, and a cortical network that includes the anterior cingulate cortex and the anterior insula is activated. However, the initial amygdala response to a fear-relevant but nonfeared stimulus (e.g., pictures of spiders for a snake phobic) disappears with conscious processing and the cortical network is not recruited. Instead there is activation of the dorsolateral and orbitofrontal cortices that appears to inhibit the amygdala response. The data suggest that activation of the amygdala is mediated by a subcortical pathway, which passes through the superior colliculi and the pulvinar nucleus of the thalamus before accessing the amygdala and which operates on low spatial frequency information.

New research indicates that a single stress episode can cause an alteration in synapse formation in the basolateral amygdala without changing dendritic length and branching. Later structural changes appear in prefrontal cortex and hippocampus as a result of single traumatic stressors, which may reflect the functional interactions with the amygdala. Together with the role of adrenal glucocorticoids and catecholamines, these results tell us how the brain is shaped by acute and repeated uncontrollable stress in ways that play a role in human anxiety disorders such as posttraumatic stress disorder (PTSD).

4.5. The Limbic System Is Involved in Psychiatric Diseases

Disorders in the limbic cortex have been linked to several psychiatric diseases (e.g., *autism, schizophrenia, and depression*). Autistic children display abnormalities in temporal lobe electroencephalographic patterns, and anatomic abnormalities have been seen in both the amygdala and hippocampal regions. The linkage between schizophrenia and limbic system dysfunction is suggested by several clinical and pathologic studies. The frontal and temporal cortices appear to be somewhat smaller in schizophrenic patients, and pyramidal neurons in the CA fields of the hippocampus are often abnormally oriented in these patients. In schizophrenic patients, there appear to be several imbalances in neurotransmitters that are selective for the limbic system. Most prominent among these imbalances are the changes in the dopamine system, which has a particularly dense projection to anterior limbic cortical regions (these regions also display decreased glucose utilization and changes in serotonergic neurotransmission). Many effective therapies for schizophrenia target the dopamine system. A linkage between depression and limbic system dysfunction has been suggested by several clinical and pathologic studies. For instance, lesions of the cingulate cortex can result in akinetic mutism.

4.6. Lesions of Subcortical Parts of the Limbic System Contribute to Dementia

Two subcortical limbic regions should be mentioned in relation to learning and memory. Some patients with relatively selective vascular lesions of the cholinergic basal forebrain display dementia, similarly patients with *Alzheimer's disease* show a loss of the cholinergic projection to the (limbic) cortex. Animal studies suggest that selective lesions of the basal forebrain projection to the limbic cortex reduce learning and memory abilities, and reestablishing this

pathway by means of transplantation of embryonic basal forebrain tissue ameliorates the lesion-induced deficits. These data suggest that the basal forebrain projection to the limbic system is a contributor to learning and memory.

The diencephalon also plays a role in learning and memory. In 1887, Korsakoff characterized a syndrome in which a severe loss of memory was present in chronic alcoholics. Patients with *Korsakoff's syndrome* display anterograde amnesia, especially for paired-association tasks, extensive retrograde amnesia for events that occurred throughout their adult life, confabulation (i.e., making up stories that are relatively plausible to attempt to cover up their memory impairments), reduced frequency of speech, little perception of their memory loss, and generalized apathy. The cause of Korsakoff's syndrome in many patients appears to be a lack of dietary thiamine (i.e., vitamin B₁), which is caused by the malnutrition that accompanies the long drinking bouts in chronic alcoholics. The syndrome consists of variable damage primarily to the medial dorsal nucleus of the thalamus, the frontal cerebral cortex, and the mammillary bodies, and the fiber tracts connecting these areas. The pivotal position of the mammillary bodies in the posterior limbic circuitry suggests that this damage is critical to Korsakoff's syndrome, but the anatomic mechanisms underlying the disease remain unresolved.

4.7. The Hippocampus Has Neural Stem Cells

Since the studies of Ramon y Cajal (1928), a central postulate in neuroscience has been that the adult brain lacks the ability to regenerate its neurons. This dogma has been challenged in the past few decades, and mounting evidence has accumulated showing the existence of *adult neurogenesis*. Until recently, neurogenesis in mammals was considered to occur only during the embryonic and early postnatal periods and to have no significant role in the adult nervous system. However, it is now accepted that neurogenesis occurs in two brain regions, the hippocampal dentate gyrus (DG) and the subventricular zone (SVZ) of the lateral ventricle. How much (if any) neurogenesis occurs normally in other human brain regions is still hotly debated. In both regions (i.e., DG and SVZ), new neurons arise from a resident population of neural progenitor cells that are maintained throughout adult life. Hippocampal neurogenesis has been shown to be required for some types of hippocampal-dependent learning. Many factors enhance hippocampal neurogenesis including hormones, growth factors, drugs, neurotransmitters, and

physical exercise as well as learning a hippocampal-dependent task. Other factors suppress hippocampal neurogenesis; these include aging, stress, high levels of glucocorticoids, and chemotherapy. Recently, much attention has become focused on the relevance of hippocampal neurogenesis to the pathophysiology and treatment of mood disorders. Altered hippocampal neurogenesis may also play a pathophysiological role in neurodegenerative disorders such as Alzheimer's disease.

Neural progenitors are found throughout the neuraxis including both neurogenic and non-neurogenic regions. When cultured *in vitro* or isolated and transplanted back into the brain, these cells can differentiate into neurons. After transplantation into adult brain, these cultured neural stem cells are capable of surviving and differentiating into both neurons and glial cells, offering hope that stem cell therapy may be used to treat a variety of neurologic and perhaps psychiatric disorders. Clinical trials with stem cell injections in Parkinson patients have been conducted, thus far with somewhat variable results. The widespread existence of endogenous neural progenitors even in non-neurogenic brain regions also offers hope that the potential of these cells may be harnessed to repair cellular injuries caused by injuries such as stroke, trauma, or neurodegenerative diseases. Furthermore, an emerging view is that astrocytes, a subset of which possibly also functions as neural progenitor cells, are critical in regulating the local environment. While obstacles remain to both approaches, stem cell-based therapies remain an area of intense clinical research interest.

4.8. The Hippocampus as a Model System Has Provided Important Insights into the Function of the Neocortex

Because of its relative simplicity, the hippocampus has often been used as a model system for investigating neuronal activity and plasticity. Because the axonal pathways of the hippocampus are very well defined, many studies have investigated the ability of axons to reinnervate areas of the hippocampus that have been denervated. These studies have also led to important insights into the role of trophic factors in neuronal reorganization. The hippocampus also has provided an excellent model in which to characterize the changes that occur in postsynaptic neurons after denervation. Together, these studies have shown that the hippocampus (brain) displays a high degree of active reorganization, an attribute that may account for its central role in learning and memory.

At the synaptic and molecular levels, the hippocampal system has been the major model in which *long-term potentiation* (LTP) has been studied. LTP is a long-lasting (i.e., hours to several days) increase in the efficiency of synaptic transmission that can be induced by a short-lasting high-frequency stimulation. This phenomenon, which was first identified in the entorhinal cortex to dentate granule cell pathway, has been subsequently demonstrated to be present in many other areas of the nervous system. It is now generally accepted that LTP is an important experimental model of the way memories are stored in the brain.

5. LIMBIC SYSTEM OVERVIEW

The limbic system is a highly interconnected group of regions that receive diverse multimodal sensory information. The amygdala and the anterior cingulate cortex primarily regulate affective behavior and visceromotor function, and the hippocampus and entorhinal cortex are predominately involved in the temporary storage of information, including the encoding of spatial relations. While having these diverse functions, the limbic cortical areas are highly interconnected and they have prominent connections with limbic subcortical, diencephalic, and brain-stem nuclei. Emotional states can significantly influence learning and memory, and the inverse is also true (i.e., elements of learning and memory such as habituation and orientation can importantly alter emotional states and arousal). Riding the wave of the rapidly progressing imaging techniques, future

studies should quickly begin to illuminate the unity and diversity of the limbic system.

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Disorders of the Limbic System

Lawrence Ver Hoef, Inga Kadish, Gregory Cooper
and Thomas van Groen

THE LIMBIC SYSTEM PLAYS A ROLE IN EPILEPSY

Many areas of the limbic system play a role in epilepsy, and one of the main areas is the hippocampus. The hypothesized relation between hippocampal formation damage and epileptic seizures dates to the early 19th century, and by 1880 Sommer had clearly identified an area of the hippocampus proper (i.e., *Sommer's sector* or area CA1) that was consistently damaged in the epileptic patients that he studied. So-called hippocampal sclerosis typically demonstrates a pattern of preferential neuronal loss and astrogliosis in the CA1 and CA4 (also known as end-folium area) subfields more than in the CA3 subfield and granule cell layer and little to no neuronal loss in the CA2 subfield. Several subsequent reports have supported the idea that, in patients with temporal lobe epilepsy, the epileptogenic focus is in the hippocampus proper in the majority of patients and that limited resection of the hippocampal focus can often abolish subsequent seizure activity. In many patients, analysis of the degree of hippocampal sclerosis (Fig. 1) compared with the frequency and severity of epileptic activity suggests that the damage to the hippocampus was present before the initial epileptic activity.

The pyramidal neurons (especially in field CA3) in the hippocampus are contributors to the initiation of epileptic seizure activity and are damaged by recurring seizures. This pattern occurs in children and adults and could perpetuate the evolution of sclerotic foci that would increase the probability and or intensity of subsequent seizures.

Other areas of the medial temporal lobe, especially the amygdala and entorhinal cortex, have also been shown to be seizure foci in a significant number of individuals.

THE LIMBIC SYSTEM ALSO PLAYS A ROLE IN ALZHEIMER'S DISEASE

In the normal course of aging, a relatively small number of neurons in the cortex, including the limbic cortex, the entorhinal cortex, and hippocampus, become dysfunctional, potentially compromising limbic memory circuits. In contrast, patients with Alzheimer's disease (AD) display extensive neuronal damage. The pathologic hallmarks of AD are intracellular neurofibrillary tangles and extracellular amyloid β plaques. Neurofibrillary tangles consist of hyperphosphorylated, twisted filaments of the cytoskeletal protein tau, whereas plaques are primarily made up of amyloid β ($A\beta$), a 39- to 43-amino-acid peptide derived from the proteolytic processing of the amyloid precursor protein (APP). When APP is sequentially cleaved by the β -secretase and the γ -secretase, the resulting breakdown product is $A\beta$; in contrast, cleavage by α -secretase does not lead to $A\beta$ production. Most cases of AD are sporadic, however approximately 5% of AD cases are familial, and these cases are most often related to mutations in the genes for APP and presenilin 1 and 2 (PS1 and PS2). The mutations alter APP metabolism such that there is an increased production of $A\beta$ (especially the longer, fibrillrogenic $A\beta42$). Together this implies a central role for aberrant APP processing in the series of pathologic changes occurring during AD,

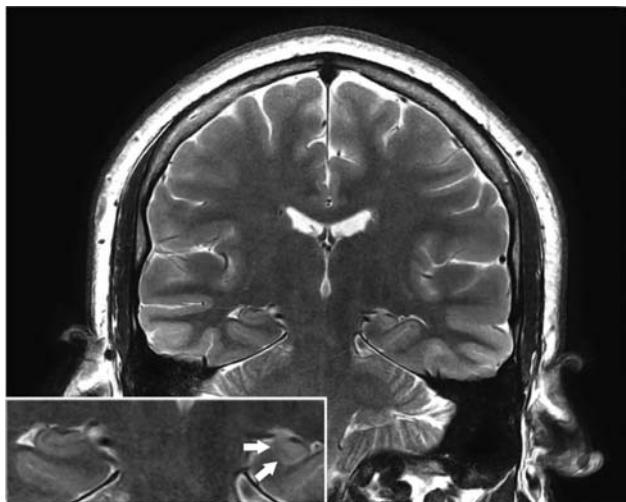


Fig. 1. A magnetic resonance imaging (MRI) coronal section through the temporal lobes of an adult with severe, long-standing epilepsy. Note the marked, unilateral atrophy of the hippocampus (arrows) in the epileptic patient.

characterized by the appearance of the typical neuritic plaques (and neurofibrillary tangles) of AD.

In the earlier reports on AD, cell loss, especially in the cholinergic basal forebrain (and in limbic cortical regions), was emphasized. The cholinergic neurons in the diagonal band of Broca and nucleus basalis of Meynert were thought to have died in Alzheimer's disease, thus significantly reducing the cholinergic input to the cortex, including parts of the limbic cortex. Subsequently, it has been demonstrated that actual cell loss is very minimal, the neurons do not die, but the somata of the affected neurons are shrunken and the neurons become dysfunctional. The discovery of the decrease in cholinergic input to the cortex in AD patients has prompted the development of acetylcholinesterase inhibitor therapy for AD; examples of these drugs are donepezil (Aricept), rivastigmine (Exelon), galantamine (Razadyne), and tacrine (Cognex). In clinical practice, however, the benefit of these medications is often modest.

The entorhinal cortices along with CA1 and the subiculum are among the earliest and most severely affected areas in AD. Damage to neurons in the subiculum would significantly affect the major outputs of the hippocampal formation, whereas the prominent loss of the layer II pyramidal neurons of the entorhinal cortex compromises the entorhinal cortex input to the hippocampus. Together, these lesions would isolate the hippocampus and thus likely will contribute to the short-term memory impairments of AD. In studies on mild cognitive impairment (MCI; i.e., possible incipient AD), one of the hallmarks in MRI

measurements is shrinkage of the entorhinal cortex, followed by shrinkage of the hippocampal formation.

Damage to other limbic areas may also contribute to many of the affective changes in later stages of the disease in Alzheimer patients. The olfactory regions of telencephalon, the cingulate cortex, and the amygdala are often compromised in Alzheimer patients, and each of these areas plays an important role in the regulation of emotions. An early clinical marker of AD, next to the onset of dementia, is the loss of smell.

THE LIMBIC SYSTEM PLAYS A ROLE IN WERNICKE-KORSAKOFF'S SYNDROME

The diencephalic part of the limbic system also plays a role in learning and memory. In 1887, Korsakoff characterized a syndrome in which a severe loss of memory was present in chronic alcoholics. Patients with *Korsakoff's syndrome* display anterograde amnesia, especially for paired-association tasks, extensive retrograde amnesia for events that occurred throughout their adult life, confabulation (i.e., making up stories that are relatively plausible to attempt to cover up their memory impairments), reduced frequency of speech, little perception of their memory loss, and generalized apathy.

Wernicke's encephalopathy is an acute, neuropsychiatric syndrome that is characterized by nystagmus and ophthalmoplegia, mental status changes, and unsteadiness of stance and gate. The disorder results from a deficiency in vitamin B₁ (thiamine). It is most often seen in people with chronic alcoholism but can also occur in a myriad of clinical settings that include gastrointestinal surgery procedures, gastrointestinal disorders associated with recurrent vomiting or chronic diarrhea, cancer and chemotherapeutic treatments, systemic diseases such as AIDS, renal diseases, thyrotoxicosis, and magnesium depletion. Furthermore, some patients may be genetically predisposed to the pathology.

In developed countries, most cases of Wernicke's encephalopathy occur in people who misuse alcohol. Other factors such as diet and existence of national programs for supplementation of foods with thiamine may have played a role in prevention of the disease. About 80% of patients with Wernicke's encephalopathy who survive develop *Korsakoff's syndrome* (a disorder that is characterized by severe memory defects, in particular, a loss of working memory that accompanies relatively little loss of reference memory). Wernicke's encephalopathy is more common in males than in females (male-to-female ratio 1.7 to 1).

Thiamine deficiency results in a diffuse decrease in cerebral glucose utilization. However, symptoms are attributed to focal areas of damage; the oculomotor signs are attributable to lesions in the brain stem affecting the abducens nuclei and the eye movement centers in the pons and midbrain. These lesions are characterized by a lack of significant destruction to nerve cells, which accounts for the rapid improvement and degree of recovery observed with thiamine repletion. Ataxia is a manifestation of damage to the cerebellum, particularly the superior vermis. The cerebellar changes consist of a degeneration of all layers of the cortex, particularly the Purkinje cells. The loss of neurons leads to persistent ataxia of gait and stance. In addition to cerebellar dysfunction, the vestibular apparatus also is affected.

The amnestic component is related to damage in the diencephalon, including the medial thalamus, and connections with the medial temporal lobes and amygdala. More specifically, the mammillary bodies sustain considerable damage. In affected areas, swelling of astrocytes is often observed with a decrease in myelinated fibers and a loss of nerve cells accompanied by activated microglia and reactive astrogliosis. Alterations in the morphology of glia represent the earliest histologic changes seen in thiamine deficiency. The slow and incomplete recovery of the memory deficits suggests that amnesia is related to irreversible structural damage.

The mechanism by which thiamine deficiency leads to damage in these specific areas is not fully understood. Proposed mechanisms include altered cerebral energy metabolism resulting from decreases in transketolase, pyruvate, and acetylcholine; diminished nerve-impulse transmission at synapses; and

impaired DNA synthesis. Variations in clinical presentations and the fact that not all patients with thiamine deficiency develop Wernicke-Korsakoff syndrome has raised the possibility that a genetic predisposition may exist in some patients.

Although amnesia and Korsakoff's syndrome involve memory deficits that are related to limbic cortex dysfunction, the two diseases have different neuropathologic and functional attributes.

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**Irene Martinez-Torres, Stephen Tisch
and Patricia Limousin**

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1. INTRODUCTION

The basal ganglia are a group of nuclei located in the diencephalon and mesencephalon. The classic concept of the basal ganglia as involved in motor control has been largely modified during the past decades on the basis of the extensive research carried out. They are known to be involved not only in motor behavior but also in cognition and emotion. They are intimately related with cortical areas and thalamus as well as with other brain-stem nuclei. Cortical information is processed by the basal ganglia in well-differentiated parallel loops, and each of these loops project back to the cortical area of origin. Although there is some segregation, cortical information from different areas is also integrated throughout the basal ganglia circuits for the selection of appropriate behaviors in relation with the environment, learning, and rewards.

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Basal ganglia dysfunction is involved in a wide range of diseases. Traditionally, basal ganglia disorders have been classified in hypokinetic and hyperkinetic disorders. Hypokinetic disorders, such as Parkinson's disease (PD), are characterized by slowness of movements, loss of movements, rigidity, and tremor. In contrast, hyperkinetic disorders (chorea, ballism, dystonia) are distinguished by an excess of movements. Although disorders of the basal ganglia were classified on the basis of the "amount" of movement, impairment of cognition and behavior are also common features of some of these diseases. The basal ganglia have also been related with neuropsychiatric disorders such as schizophrenia and obsessive-compulsive disorder supporting their role in emotional functions.

The resurgence of functional neurosurgery and in particular the development of deep brain stimulation for certain of these conditions has allowed confirmation in humans of some of the data found in animal studies, and has contributed to our understanding of neuronal activity in pathologic and physiologic conditions.

2. ANATOMY OF THE BASAL GANGLIA

The basal ganglia comprise four major nuclei: the striatum (caudate nucleus and putamen), the globus pallidus (GP, internal and external segment), the subthalamic nucleus (STN), and the substantia nigra (SN, pars compacta and pars reticulata) (Fig. 1). The striatum is the main input structure. It receives massive afferents from the entire cerebral cortex as well as from the thalamus and in a lesser degree from the dorsal raphe nucleus and the amygdala. The output nuclei are the internal segment of the globus pallidus (GPi) and the pars reticulata of the substantia nigra (SNr). The striatum that uses gamma-aminobutyric acid (GABA) as a neurotransmitter projects directly or indirectly, via the external segment of the GP (GPe) and the STN, to the output nuclei, which in turn project to the thalamus.

The globus pallidus is divided into the internal and external segment (GPi and GPe) by the internal medullary lamina. Although GPe and GPe share similar morphology and a common neurotransmitter, GABA, they are functionally distinct. The GPe is one of the output nuclei of the basal ganglia, whereas the GPe could be considered as a modulator nucleus of the activity of the basal ganglia. Similarly, the SN consists of two major subnuclei, the pars compacta (SNC) and the SNr. These two parts share similar inputs from other basal ganglia nuclei and have mostly different outputs and are neurochemically distinct. The SNr uses GABA as neurotransmitter, whereas the SNC uses dopamine. Because of these differences, they will be considered separately in this chapter. The STN uses glutamate as neurotransmitter

and can be considered as both modulator and input structure.

Cortical information is processed in a segregated topographic manner that is maintained along the whole axis of the basal ganglia. Three main territories can be identified within the nuclei: sensorimotor, associative, and limbic. Within the sensorimotor territory, it is also possible to identify a body map (somatotopy), analogous to the cortical homunculus, and the information for the different body parts is also processed in parallel. Although there is a high degree of segregation of cortical information, convergence also exists within the basal ganglia.

2.1. The Input Nucleus of the Basal Ganglia: The Striatum

2.1.1. STRIATAL ORGANIZATION

The striatum is the major input structure of the basal ganglia and comprises the caudate, putamen, and accumbens (ventral striatum) nuclei. The caudate nucleus is medial to the putamen and is separated from it by the internal capsule.

Anatomic and biochemical studies have revealed a macroscopic organization of the striatum in two compartments: the patch compartment, or striosomes, and the matrix. This compartmental architecture is fundamental to understand some of the functions of the striatum. Limbic and paralimbic cortices project mainly to the striosomes, whereas sensorimotor and associative cortex project preferentially to the matrix. Different cortical layers also project preferentially to the striosomes (deeper cortical layers) or matrix (more superficial cortical layers).

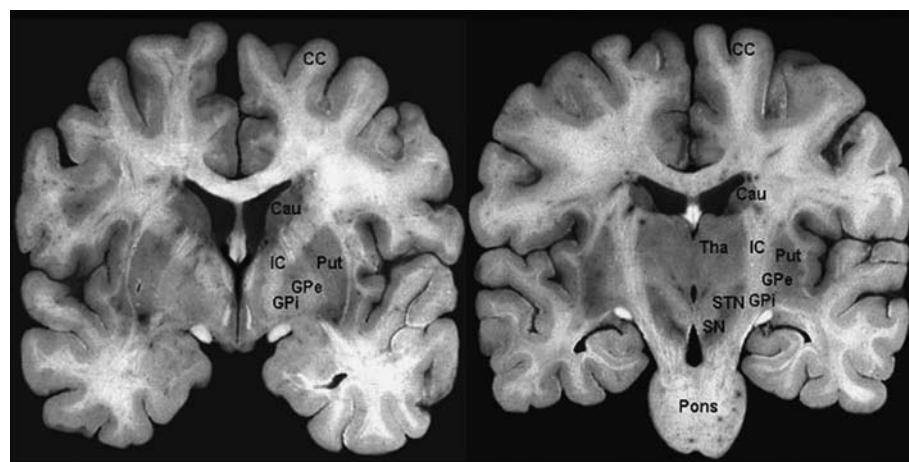


Fig. 1. Brain slices in the coronal plane showing the basal ganglia nuclei. Cau, caudate nucleus; CC, cerebral cortex; GPe, globus pallidus externus; GPI, globus pallidus internus; IC, internal capsule; Put, putamen; SN, substantia nigra; STN, subthalamic nucleus; Tha, thalamus.

The principal neurons of the striatum are the medium spiny neurons (95% of the neurons in the striatum in rodents and 75% to 80% in primates). They use GABA as a neurotransmitter and project to output nuclei of the basal ganglia. They also emit a dense local axon collateral system that innervates other spiny neurons and the striatal interneurons. They receive glutamatergic afferents mainly from the cortex and thalamus and dopaminergic afferents from the SNC. Convergence of cortical and thalamic information occurs at the level of individual spiny neurons in the striatum.

The interneurons, or aspiny neurons (because of the absence or rarity of spines in their dendrites), have been classified based on morphologic, neurochemical, and physiologic differences into one population of cholinergic cells and three distinct types of GABAergic interneurons according to the coexpression of parvalbumin, calretinin, or somatostatin/nitric oxide/neuropeptide Y. Interneurons receive excitatory inputs from the cortex and the thalamus and exert a strong inhibitory feedforward effect over the spiny neurons. Spiny neurons are also inhibited in a weaker manner by the local axon collaterals. Cholinergic neurons are also known as TANs (tonically active neurons) and were shown to respond to visual and auditory cues that predict saliency or reward in operant tasks. More recently, cholinergic interneurons have been pointed to as key mediators of dopamine-dependent striatal plasticity and learning and as modulators of excitatory cortical inputs to the striatum.

2.1.2. AFFERENTS TO THE STRIATUM

2.1.2.1. Corticostriatal Projections. The striatum receives glutamatergic afferent from all cortical areas. These corticostriatal projections are topographically organized and project to three distinct regions of the striatum: the sensorimotor, the associative, and limbic striatum. The sensorimotor territory comprises the dorsolateral sector of the postcommissural portion of the putamen and the dorsolateral rim of the head of the caudate nucleus; it receives projection from primary motor cortex, somatosensory cortex, premotor cortex, supplementary motor area, and cingulate motor area. A recent study revealed that the sensorimotor striatum also received axon collaterals from corticofugal axons that descend toward the brain stem. Electrophysiologic studies have shown that neurons located in the sensorimotor striatum respond to passive and active movements of the limbs, and a well-defined somatotopic organization has been

described, with the leg being dorsal and the trunk, arm, and head ventral. The associative territory comprises large part of the putamen rostral to the anterior commissure and most of the head, body, and tail of the caudate nucleus; it receives projections from associative cortices in frontal, parietal, and temporal lobes. The limbic striatal territory comprises the nucleus accumbens, the deep portion of the olfactory tubercle, and the most ventral part of the caudate nucleus and the putamen; it receives projections from the limbic and paralimbic cortex, the amygdala, and the hippocampus.

2.1.2.2. Nigrostriatal Projections. The striatum receives dopaminergic afferents from the SNC. Other sources of dopaminergic inputs come from the retrorubral region (A8) and the ventrotegmental area (VTA). The VTA projects mainly to the ventral striatum as well as limbic forebrain areas, whereas the retrorubral region and SNC project to the dorsal striatum.

Dopaminergic nigral neurons make synaptic contacts with neck of the dendrite spines, but only in those spines that also receive cortical input. Such synaptic organization allows dopamine to modulate the excitatory effect of corticostriatal projections.

2.1.2.3. Thalamostriatal Projections. The striatum receives major thalamic glutamatergic afferents from the centromedian/parafascicular (CM-Pf) complex of the thalamus. The CM nucleus projects mainly to the putamen (sensorimotor striatum) and receives afferents from the motor cortex and GPi. The Pf nucleus projects to the caudate nucleus (associative striatum) and the pallidum, and its afferents come from the premotor cortex. The ventromedial part of the Pf nucleus also projects to the limbic striatum. Thalamic afferents project to the matrix, and synapses are located on the distal portion of the dendrite of spiny neurons, allowing modulation of corticostriatal inputs.

2.1.2.4. Subthalamic Projections. The inputs from the STN are glutamatergic and are also segregated in the three main domains. Subthalamic projections are scarce and exert an *en passant* excitatory effect over striatal neurons.

Other inputs to the striatum are serotoninergic projections from the midline raphe nuclei and norenergic from the locus coeruleus.

2.1.3. STRIATAL EFFERENTS

The medium spiny neurons receive most of the striatal afferents and are also the cells that project outside the striatum. Spiny neurons can be divided into two subgroups according to their neurochemical features: spiny neurons containing enkephalin and expressing predominately D2 subtype of dopamine receptors that project to the GPe (indirect pathway), and spiny neurons containing substance P (SP) and dynorphin and expressing mainly D1 subtype receptors that project to the SNr and the GPi (direct pathway). Efferents to the pallidum are mainly from the putamen and convey sensorimotor information. Those to the SNr originate mainly in the caudate nucleus and convey information from the associative cortex.

2.2. Control Nuclei of the Basal Ganglia: The Globus Pallidus External Segment and the Subthalamic Nucleus

2.2.1. GLOBUS PALLIDUS EXTERNAL SEGMENT

The GPe receives massive GABAergic afferents from the striatum and glutamatergic afferents from the STN. Striatal and subthalamic afferents form parallel bands to the internal lamina in both segments of the pallidum. Both striatum and STN send convergent fibers to pallidal neurons suggesting that cortical information received by these two structures could be integrated at the level of single pallidal neurons. Other inputs to the GPe come from the cerebral cortex, intralaminar thalamic nuclei (CM/Pf), GPi, SNC, raphe, and pedunculopontine nucleus (PPN).

The majority of the projections of the GPe end at the STN (indirect pathway), GPi/SNr, and striatum. A small number of GPe neurons innervate the dorsal thalamus, inferior colliculus, and the PPN. GPe also send projections to the reticular nucleus of the thalamus, which could provide a route for conveying basal ganglia influences to most of the thalamic nuclei. Reciprocal loops exist between the GPe-striatum and the GPe-STN. The GPe has been classically seen as an indirect link between the striatum and the output nuclei of the basal ganglia. However, the existence of a massive inhibitory projection from the GPe to the GPi/SNr places the GPe in an essential position to directly control the output stations of the basal ganglia.

2.2.2. SUBTHALAMIC NUCLEUS

The STN can be considered as an input and control nucleus of the basal ganglia. It receives massive projections from the primary motor cortex, supplementary

motor area, and premotor cortex that terminate in the sensorimotor STN. These cortico-subthalamic projections constitute the fastest pathway by which cortical information reaches the basal ganglia and is known as the hyperdirect pathway. Other important input to the STN comes from the GPe with which it forms a reciprocal loop and constitutes the indirect pathway from the striatum to the output nuclei. The STN also receives projections from the intralaminar thalamic nuclei keeping a topographic organization where the CM nucleus projects to the sensorimotor STN and the Pf nucleus innervates its associative and limbic territories. Other inputs come from the SN and various brain-stem nuclei (the dorsal raphe nucleus and the PPN).

The dorsolateral part of the STN is the largest portion of the nucleus and corresponds with the sensorimotor territory. Neurons in this area change their discharge rate during movements. A representation of the body map has also been delineated: the leg is dorsal, the face ventral, and the arm is in-between. Associative cortical areas and frontal eye fields project to the ventromedial part of the STN (associative territory). The medial tip of the STN is connected with limbic structures and is considered the limbic territory.

The major efferent projections from the STN project to both segments of the globus pallidus in a topographic arrangement. These projections form parallel bands in the GPe and GPi. Part of the subthalamonigral projections that reach the SNr are involved, together with the caudatonigral projections, in the control of saccadic eye movements. Some of the axons of subthalamonigral neurons located in the ventromedial part of the STN ascend and synapse the neurons in the SNC, comprising one of the mechanisms for the control of dopamine release. STN also sends scant projections to the striatum with a topographic organization and to the PPN and ventral tegmental area.

2.3. The Output Nuclei of the Basal Ganglia: The Globus Pallidus Internal Segment and the Substantia Nigra Pars Reticulata

The output nuclei of the basal ganglia are the GPi and the SNr. Both structures have similar connections and differ in their functions and topographic organization. They receive cortical information processed by the striatum, both directly and indirectly, through the GPe and STN. GPi receives more prominent projections from the postcommissural putamen, which is the striatal territory that receives inputs from

the sensorimotor cortex. Projections from the caudate nucleus and the rostral precommissural putamen, the striatal territory that receives input from the associative cortex, terminate mainly in the SNr, suggesting that this nucleus is more concerned with associative/cognitive tasks whereas GPi is more involved in motor control.

2.3.1. GLOBUS PALLIDUS INTERNAL SEGMENT

The GPi receives inputs from the striatum (direct pathway), STN (indirect pathway), and GPe. Other inputs come from the intralaminar thalamic nuclei, the dorsal raphe nucleus, the PPN, and from the SNC.

As it has been mentioned above, the projections to the GPi are also organized in parallel functional domains. The sensorimotor area of the GPi is localized in the postero lateral of the GPi, displaying a somatotopic arrangement, with the leg being dorsal, the head ventral, and the arm in-between. The associative territory is localized in the dorsal one-third of the GPi and the limbic in the medial tip. Afferents from the GPe and STN project onto the same neurons in the GPi. Reciprocal connections exist between GPi and GPe.

Neurons in the GPi are GABAergic and fire spontaneously at high frequencies without pauses, which entails a tonic inhibition of thalamic target that is released by the activation of the direct pathway facilitating thus thalamocortical projections. The pattern of arborization of pallidal neurons is different within each target. They project to the ventral anterior (VA, pars principalis) and ventral lateral (VL, pars oralis) thalamic nuclei onto the thalamocortical neurons and thalamic interneurons, suggesting that they exert a double inhibition onto the thalamic projection neurons, one directly and other by inhibiting the excitation that the interneurons exert on them. Pallidothalamic projections to the VA/VL give off collaterals to the CM nucleus in primates, which in turn projects back to the striatum forming an ancillary subcortical loop (striatum-GPi-CM-striatum) that conveys sensorimotor information. The existence of a similar parallel loop involving the Pf nucleus has been proposed to convey associative type information. Other output structures receiving projections from the GPi are the habenula, which is involved in limbic functions, and the PPN.

2.3.2. SUBSTANTIA NIGRA PARS RETICULATA

The SNr receives major GABAergic projections from the medium spiny neurons of the striatum, which make synapse with the distal portions of the dendrites of the SNr cells. The GPe provides an

additional source of GABAergic projections to the SNr. GPe neurons synapse with the proximal dendrites of the nigral cell suggesting that this nucleus may have a significant impact on the discharge of SNr neurons. The pallidonigral neurons receive inputs from the striatum. Thus, in addition to the direct striatonigral pathway, which provides an inhibitory input to the SNr, the striatum exerts an excitatory influence to the SNr via striato-pallido-nigral pathway. The SNr also receives inputs from different parts of the STN (indirect and hyperdirect pathway). Striatonigral, pallidonigral, and subthalamonigral inputs are topographically organized and converge onto the same SNr output neurons.

The majority of the SNr neurons are projection neurons and express the neurotransmitter GABA. They also form an axon collateral network, exerting an additional function of interneurons.

SNr neurons and nigral afferents are topographically organized following a laminar arrangement in an onion-like manner. The lateral half of the SNr processes information coming from sensory and motor cortical areas. The medial part of the SNr is innervated by striatal subterritories related to prefrontal and limbic cortical areas. Efferent neurons keep the topographic subdivisions and their axons are highly collateralized, and the same nigral neuron projects to the same topographic region in the thalamus, superior colliculus, and pontine reticular formation.

The SNr send GABAergic projections to the VA (pars magnocellularis), VL (pars medialis), and dorsomedial (MD, pars paralaminaris) thalamic nuclei, particularly to the thalamocortical neurons, the superior colliculus, and mesopontine tegmentum. SNr also projects to dopaminergic neurons of SNC, predominately to those projecting to striatum efferents to SNr.

3. INTRINSIC CIRCUITS OF THE BASAL GANGLIA

Projections from the striatum reach the output nuclei (GPi/SNr) via two different pathways: the direct and indirect pathways. In the direct pathway, the spiny neurons (D1 receptor/SP/dynorphin) project monosynaptically onto the GPi/SNr, which project to the thalamus, facilitating thalamocortical projections and cortical initiated movements. In the indirect pathway, spiny neurons (D2 receptor/ENK) project onto the GPe, which connect with the STN that in turn project to the GPi/SNr. The STN uses

glutamate as a neurotransmitter and therefore exerts an excitatory effect on the GPi/SNr that in turn inhibit thalamocortical projections. Dopamine appears to modulate the activity over the two pathways. It excites the D1 receptors of the direct pathway and inhibits D2 receptors in the indirect pathway. The overall effect of dopamine on the striatum is to reduce the basal ganglia inhibitory output, leading to an increase of thalamocortical projections (Fig. 2).

The basal ganglia nuclei also participate in several subsidiary circuits: (1) the CM/Pf thalamic nuclei-striatum-GPi-CM/Pf, which is probably a positive feedback loop leading to increase striatal neuronal activity; (2) CM/Pf-STN-GPi-CM/Pf circuit, which is probably a negative loop leading to reduced neuronal activity; (3) STN-GPe-STN circuit, which is an excitatory-inhibitory loop with autostabilizing characteristics; and (4) the STN-GPe/GPi dual projections. Another important connection is the direct cortical projection to the STN, which may be important in synchronizing oscillatory activity in the cortex, STN and pallidum (Fig. 3).

Apart from the ascending projections to the thalamus, the GPi/SNr also project to brain-stem nuclei such as PPN and midbrain extrapyramidal area.

These nuclei send reciprocal excitatory (cholinergic and glutamatergic, respectively) ascending projections to the basal ganglia and thalamus and descending projections to the spinal cord. The PPN appears to be involved in a variety of behavioral as well as locomotor functions. Some recent preliminary results suggest that PPN may be a promising target for deep brain stimulation (DBS) in Parkinson's disease patients with gait and balance problems resistant to levodopa.

In general, disorders of the basal ganglia can be classified as hypokinetic (e.g., Parkinson's disease) and hyperkinetic (Huntington's disease, dystonia, ballism). They are associated with an increased or decreased basal ganglia output respectively. In parkinsonism, loss of dopamine leads to a shift of the balance to the indirect pathway with increased excitation of GPi/SNr neurons and greater inhibition of thalamocortical projections with a subsequent development of akinesia (loss of movement) and bradykinesia (slowness of movement) among other symptoms. By contrast, in hyperkinetic disorders, the basal ganglia output is reduced leading to a disinhibition of the thalamocortical projections and development of involuntary movements.

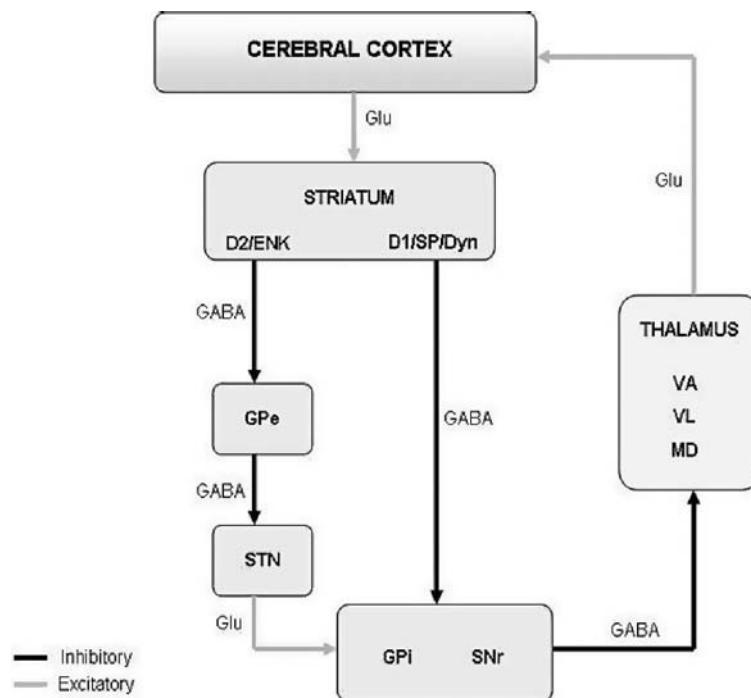


Fig. 2. Schematic representation of the direct and indirect pathways between the striatum and the GPi/SNr. D1, D1 subtype of dopaminergic receptor; D2, D2 subtype of dopaminergic receptor; Dyn, dynorphin; ENK, enkephalin; GABA, gamma-aminobutyric acid; Glu, glutamate; GPe, globus pallidus externus; GPi, globus pallidus internus; MD, mediodorsal thalamic nucleus; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; VA, ventral anterior thalamic nucleus; VL, ventral lateral thalamic nucleus.

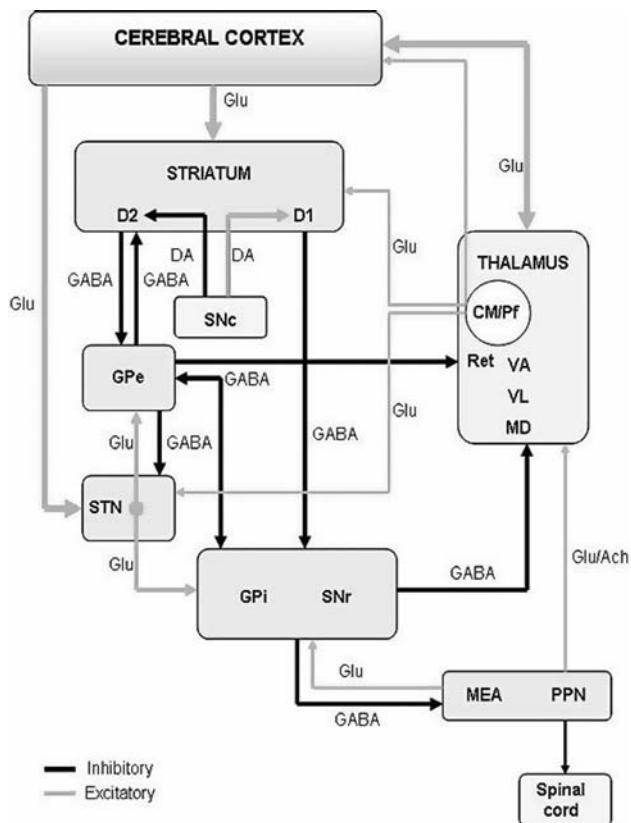


Fig. 3. Schematic representation of the organization of the basal ganglia-thalamocortical circuits. Ach, acetylcholine; CM/Pf, cenmedian and parafascicular thalamic nuclei; D1, D1 subtype of dopaminergic receptor; D2, D2 subtype of dopaminergic receptor; DA, dopamine; Dyn, dynorphin; ENK, enkephalin; GABA, gamma-aminobutyric acid; Glu, glutamate; GPe, globus pallidus externus; GPi, globus pallidus internus; MD, mediodorsal thalamic nucleus; MEA, midbrain extrapyramidal area; PPN, pedunculopontine nucleus; Ret, reticular thalamic nucleus; SNC, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; VA, ventral anterior thalamic nucleus; VL, ventral lateral thalamic nucleus.

4. BASAL GANGLIA-THALAMOCORTICAL CIRCUITS

Traditionally, the basal ganglia were seen as structures that funneled the information originating in distinct cortical areas and then projected back to the primary motor cortex. In 1986, Alexander et al. on the basis of the anatomic and physiologic findings accumulated described the existence of five circuits between cortical areas and the basal ganglia: the motor circuit, the oculomotor circuit, two prefrontal circuits (the dorsolateral prefrontal circuit and the lateral orbitofrontal circuit), and the limbic circuit

(Table 1). The designation of the circuits was made according to its cortical area of origin and termination (Fig. 4). The segregated organization of these loops has been given further support by studies using retrogradely transported virus particles. These studies have also resulted in the identification of two additional circuits, one originating from one of the visual areas of the inferotemporal cortex (area TE) and another from the posterior parietal cortex. More recently, studies using transneuronal transport of rabies virus have confirmed the segregation pattern from the cortex to the third-order neurons in the GPe, STN, or striatum and the existence of an open loop between the primary motor cortex and the ventral putamen. This open loop may allow interaction between the limbic system and motor functions.

All circuits share similar characteristics, they originate in specific cortical areas, pass through separated portions of the basal ganglia and thalamus, and end in the cortical area of origin. Within each circuit, the information is processed following the direct and indirect pathways that link the striatum with the output nuclei. However, this last organization is less clear in the limbic circuit.

The circuits maintain a clear topographic organization of inputs and outputs through the basal

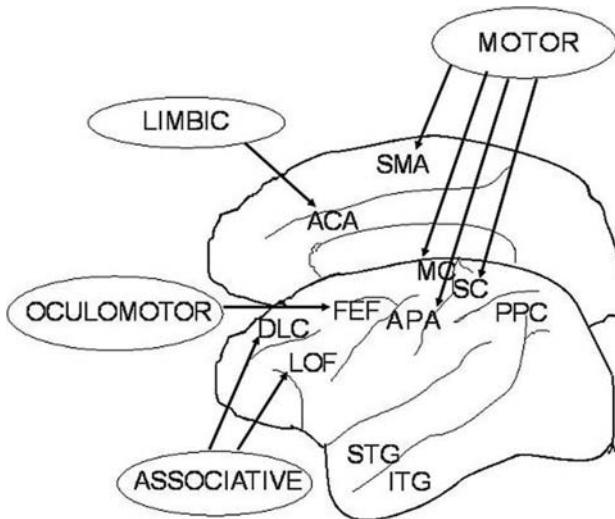


Fig. 4. Schematic representation of the cortical areas related to each basal ganglia-thalamocortical circuit. ACA, anterior cingulated area; APA, arcuate premotor area; DLC, dorsolateral prefrontal cortex; FEF, frontal eye fields; ITG, inferior temporal gyrus; LOF, lateral orbitofrontal cortex; MC, motor cortex; PPC, posterior parietal cortex; SC, somatosensory cortex; SMA, supplementary motor area; STG, superior temporal gyrus.

Table 1
Basal Ganglia–Thalamocortical Circuits

Circuit	Motor	Oculomotor	DLC	LOF	Limbic
Cortical areas	MC, SMA, SC, APA	FEF, DLF, PPC	DLC, PPC, APA	LOF, STG, ITG, ACA	ACA, MOF, HC, EC, STG, ITG
Striatum GPi/SNr	Put	Cau-body	Cau-head dl	Cau-head vm	VS
	dl-GPi	cdm-GPi	ldm-GPi	mdm-GPi	rl-GPi, VP
	cl-SNr	vl-SNr	rl-SNr	rm-SNr	rd-SNr
Thalamus	VLo	l-VAmc	VApC	m-VAmc	pm-MD
	VLM	MDpl	MDpc	MDmc	
Cortical areas	SMA	FEF	DLF	LOF	ACA
Function	Control of direction and scaling of movement. Sequential movement.	Saccadic eye movements	Working memory, execution function	“Switching behavioral set”	Motivational behavior, emotion
Dysfunction	PD, dystonia, chorea, ballism, TS	HD PD	TS	TS	TS OCD

ACA, anterior cingulate area; APA, arcuate premotor area; Cau, caudate nucleus; DLC, dorsolateral prefrontal cortex; EC, entorhinal cortex; FEF, frontal eye fields; GPe, globus pallidus externus; GPi, globus pallidus internus; HC, hippocampal cortex; HD, Huntington's disease; ITG, inferior temporal gyrus; LOF, lateral orbitofrontal cortex; MC, motor cortex; MDpl, mediodorsal nucleus pars paralamellaris; MDmc, mediodorsal nucleus pars magnocellularis; MDpc, mediodorsal nucleus pars parvocellularis; MOF, medial orbitofrontal cortex; OCD, obsessive-compulsive disorder; PD, Parkinson's disease; PPC, posterior parietal cortex; Put, putamen; SC, somatosensory cortex; SMA, supplementary motor area; SNr, substantia nigra pars reticulata; STG, superior temporal gyrus; TS, Tourette's syndrome; VAmc, ventral anterior pars magnocellularis; VApC, ventral anterior pars parvocellularis; VLM, ventral lateral pars medialis; VLo, ventral lateral pars oralis; VP, ventral pallidum; VS, ventral striatum; cl, caudolateral; cdm, caudal dorsomedial; dl, dorsolateral; l, lateral; ldm, lateral dorsomedial; m, medial; mdm, medial dorsomedial; pm, posteromedial; rd, rostrodorsal; rl, rostralateral; rm, rostromedial; vm, ventromedial; vl, ventrolateral.

ganglia. The segregation is such that further channels within circuits can be found. For example, in the motor circuit, which is the best studied, the information from different cortical areas (somatosensory, motor, and premotor cortices) is processed in parallel. These channels within the motor circuit are subdivided in somatotopic subchannels representing each body part. Furthermore, neurons in the putamen that represent a single body part respond to different characteristics of the movement: some respond to preparation of the movement, others are movement related, and others are specific to the direction of the movement. Therefore, a deeper level of segregation has been suggested within the somatotopic subchannels on the basis of the pattern of response of the neurons.

Despite the high level of segregation, convergence also exists. This idea is supported by the comparison of the large number of corticostriatal versus the much smaller number of striatal and pallidal output neurons. Anatomic and electrophysiologic studies have demonstrated that convergence of information

occurs within distinct territories of the same nuclei and at the level of single cells.

4.1. The Motor Circuit

The motor circuit originates from the somatosensory, motor, and premotor cortex and projects onto the postcommissural putamen. Two subloops can be identified according to the cortical area of origin, one projecting onto the lateroventral putamen and the other onto the dorsomedial, which are involved in the processing of simple and complex motor tasks, respectively. Between these two distinct areas of the somatosensory striatum, there is an area where the information of both subloops overlaps, allowing integration of information originating from different cortical sources. A somatotopic distribution of the cortical afferents is found in both areas of the putamen leading to a further segregation of the circuit. Sensorimotor subdivisions of the putamen project to the corresponding territories in the GPi/SNr. GPi projects to the VL thalamic nucleus and SNr to VA and mediodorsal thalamic nuclei (MD). Whereas topographic segregation of

information is the predominant processing modality performed along the striatopallidal pathway, which is mostly involved in sensorimotor processing, convergence of information seems to be the main processing modality along the striatonigral pathway, which is mostly involved in associative processing.

4.2. The Oculomotor Circuit

The oculomotor circuit is centered on the body of the caudate that receives inputs from the frontal eye fields that also project onto the superior colliculus. The body of the caudate nucleus projects to the ventrolateral SNr and dorsomedial GPi. SNr projection branching neurons send collaterals to the thalamus (VA and MD nuclei) and to the superior colliculus. From the thalamus, the information is sent back to the frontal eye fields. Neurons in the ventrolateral part of the SNr respond to visual passive stimulation, fixation of gaze, and both visually triggered and memory-contingent saccadic eye movements. Another circuit that contributes to control of saccadic eye movements is constituted by frontal eye fields-STN-SNr. SNr is known to exert a tonic GABAergic inhibition on the superior colliculus neurons. During saccadic eye movements, this inhibition is suppressed by striatonigral GABAergic projections. The STN exerts an excitatory influence on the nigrocollicular neurons modulating the striatal activity.

4.3. The Associative Circuits

The *dorsolateral prefrontal* circuit originates from the dorsolateral prefrontal cortex and projects onto the dorsolateral caudate nucleus that directs the information to the dorsomedial one-third of the GP and to rostral portions of the SNr. GPi projects to the VA (parvocellular portion) and SNr to the mediodorsal thalamic nucleus (MD). These output structures project to different areas within the thalamus. This circuit may participate in spatial memory processes.

The *orbitofrontal* cortex projects to the ventromedial caudate nucleus that projects to the rostromedial portion of the SNr and dorsomedial sector of GPi. The latter nuclei project in turn to MD and VA nuclei of the thalamus. The thalamus directs its projections to the orbitofrontal cortex. The orbitofrontal circuit is considered to play a role in “switching behavioral set.”

4.4. The Limbic Circuit

In the limbic circuit, the anterior cingulate cortex projects to the ventral striatum (nucleus accumbens and olfactory tubercle). The ventral striatum also

receives inputs from medial orbitofrontal cortex, temporal lobe, as well as from limbic structures (hippocampus, amygdala, and entorhinal and perirhinal cortices). The ventral striatum projects to the ventral pallidum, rostral-lateral GPi, and to the rostrodorsal SN, which, in turn, project to the thalamus (MD) that projects back to the anterior cingulate and medial orbitofrontal cortices closing the limbic circuit. The limbic circuit participates in motivational behaviors and emotion.

5. FUNCTION AND PHYSIOLOGY OF THE BASAL GANGLIA

Research in recent years has challenged the traditional view of basal ganglia as only involved in control of movement. Anatomic studies have demonstrated the connection between basal ganglia and cortical areas concerned with cognition. The activity of the neurons within the basal ganglia nuclei is more related to cognitive or sensory tasks than to motor function. Finally, some lesions in the basal ganglia produce cognitive and sensory disturbance, sparing the motor function.

5.1. Motor Function

Despite the recent interest in nonmotor function of the basal ganglia, most of the research has focused on motor aspects of basal ganglia physiology.

Microelectrode studies in primates have allowed us to describe the neuronal activity within the basal ganglia nuclei. Neuronal activity is defined by firing rate, pattern of discharge, and the degree of synchronization and frequency of oscillation of neuronal populations. Variation in these parameters can influence the function of the basal ganglia in normal and pathologic conditions. The firing rates of neurons vary by nuclei. At rest, striatal neurons show a low frequency rate; GPi and SNr neurons show a high rate and tonic discharging pattern. In the GPe, two types of activity have been recognized: neurons with a pausing and slightly lower discharge rate than GPi neurons, and other neurons with very low spontaneous rate with occasional high-frequency bursts. STN neurons fire tonically at medium frequencies (20 to 30 Hz). Thus, at rest, basal ganglia nuclei neurons discharge tonically, independently, and mainly in a nonoscillatory way.

These studies have demonstrated the existence of neurons within the basal ganglia nuclei that change their firing rate (increase or decrease) in relation to movement. A clear somatotopic organization within

the sensorimotor territory of the nuclei has been delineated. Pallidal neurons respond selectively to single joint and direction of movement. Some studies have also found specificity related with some parameters of the movement such as amplitude and velocity. The majority of pallidal neurons increase their firing rates in response to movement thus leading to an inhibition of thalamocortical projections and only a small number of cells decrease their firing rate. On the basis of these findings, Mink et al. proposed the center-surround model, where the primary role of the basal ganglia is to focus selection of desired movement and to inhibit competing movements. In this model, the direct pathway constitutes the excitatory center and the indirect pathway is proposed to provide the inhibitory surround suppressing competing motor programs. Electrophysiologic studies have also revealed that neuronal activity of the basal ganglia structures occurs relatively late to be involved in execution or planning of movements. Initiation of movement is most likely to occur at cortical levels.

Neurons in oculomotor circuit do not change their discharge in response to all saccades but appear to be activated in response to attractive targets in the environment or to remembered points in visual space. This suggests that basal ganglia will respond most likely to facilitate movement in particular circumstances or contexts rather than to operate in a particular type of movement. In this line, Brotchie et al. demonstrate that GP appears to be more involved in movements that are predictable and well practiced. They found that pallidal neurons discharge in a phasic way during a sequential movement task. They suggested that phasic discharge may be the “internal cue” to switch from one movement to another, particularly when the second movement is predictable and automatic (after learning). Further studies have also pointed to the participation of the GPi in sequential movements. Other regions of the striatum different to the motor territory respond to environmental cues in preparation of the movement.

Further studies have demonstrated that distinct parts of the striatum respond to visual stimuli (tail of caudate and ventral putamen), to visual stimuli of emotional significance (ventral striatum), or to environmental events that are cues for behavioral responses (head of caudate). The dopaminergic nigrostriatal neurons also show responses that are context dependent, particularly they respond in relation to reward or predicted reward after learning.

More recently, studies have emphasized the role of neuronal oscillations and synchrony in pathologic

conditions such as Parkinson’s disease. However oscillatory activity, although weak, also exists in the physiologic state. According to the frequency, oscillatory activity can be divided in different bands. Oscillations between 8 and 30 Hz are the best documented in human striatum, GPi, and STN. This band is subdivided into 8- to 13-Hz and 14- to 30-Hz bands. Oscillations in the latter range are known as beta band. Suppression in the beta band is seen prior to voluntary movements in normal conditions. An augmentation of the power in this band occurs when a pre-prepared movement requires cancellation. Beta activity in the cortex behaves in a similar manner to that in the basal ganglia. These findings suggest that beta band oscillatory activity may play a role in the normal function of the basal ganglia and that its attenuation may be necessary for generation of motor behaviors.

Alterations in the degree of oscillations and pattern of discharge are seen in pathologic conditions. Parkinson’s disease is associated with an abnormal increase in discharge rate, a greater tendency of neurons to discharge in burst, and an increase in oscillatory and synchronized activity. The direct connection between the cortex and the STN as well as the basal ganglia and thalamus may serve to predispose the circuit to synchronize oscillatory activity. Hyperkinetic disorders such as chorea and dystonia are associated, in general, with a decrease in firing rate and increase of synchronization.

5.2. Cognitive and Behavior Functions

On the basis of anatomic observation, it is apparent that cortical areas such as dorsolateral prefrontal cortex, the lateral orbitofrontal cortex, and the anterior cingulate/medial orbitofrontal cortices are connected with the basal ganglia. These frontal regions are involved in planning, working memory, rule-based learning attention, and other aspects of higher executive function. More recently, a new output from the basal ganglia to the area TE of inferotemporal cortex has been identified. This cortical area participates in higher-order visual functions and in visual working memory.

The role of basal ganglia in cognition and behavior is supported by electrophysiologic, functional imaging, and clinical studies.

Electrophysiologic studies in monkeys have stressed that the majority of the neurons of the output nuclei do not respond to movement. These neurons are located within regions of the GPi and SNr that project to prefrontal cortices. Recordings of single

neurons in trained primates showed neurons in the SNr that change their activity during the cue and delay periods of the tasks but not during the movement period. Other studies have revealed that the responses of striatal neurons depend strongly on the reward contingencies of the task. Inactivation of the caudate and anterior striatum of primates leads to deficits in learning sequences. Moreover, some studies have shown that certain outputs from the GPi participate in tasks involving the use of working memory. A recent study in primates demonstrated that the inactivation of the GPe using a GABA antagonist induced stereotyped behaviors when performed in the limbic part of the GPe and attention deficit and/or hyperactivity when performed in the associative territory of the GPe. Functional imaging studies have demonstrated the activation of caudate nucleus during learning of new sequences and the participation of GPi in planning and spatial working memory.

Clinical studies support the role of the basal ganglia in cognition. Lesions or diseases involving the striatum in humans (Parkinson's disease, Huntington's disease) as well as of the output nuclei are correlated with cognitive impairment. In Parkinson's disease, which is characterized by a reduction in the dopaminergic nigrostriatal inputs, deficits in attentional set shifting, working memory, planning, and problem solving can be identified. Bilateral lesions of the SNr produce deficits in working memory, visual hallucinations, and other neurologic symptoms. Lesions in the pallidum can also produce cognitive deficits, particularly in implicit learning, compulsive behaviors, and "psychic" akinesia.

Overall, there is growing evidence that alterations of the basal ganglia occur with neuropsychiatric disorders, such as depression, obsessive-compulsive disorder, Tourette's syndrome, autism, and attention deficit disorder. Deep brain stimulation (DBS) of different nuclei of the basal ganglia are currently being explored to treat some of these disorders with encouraging results.

6. PHARMACOLOGY OF THE BASAL GANGLIA

Four main neurotransmitters act in the basal ganglia: glutamate, GABA, dopamine, and acetylcholine (Ach). Cortical and thalamic inputs to the striatum are glutamatergic as well as thalamocortical projections. With the exception of the STN, which also uses glutamate, the rest of the basal ganglia nuclei use GABA as neurotransmitter. Dopamine

has an important modulatory effect on the striatum, and the levels of dopamine are crucial to determine the output activity of the basal ganglia. The role played by acetylcholine is far from being ancillary, and it might influence striatal output by neuromodulating corticostriatal glutamatergic projections. All the basal ganglia nuclei also receive serotonergic input from the rostral raphe nuclei in the midbrain and upper pons. The major target for the serotonergic projections is the medium spiny neurons. Animal studies suggest that serotonin may exert a tonic inhibitory effect on striatal glutamatergic input and on stimulated dopamine release.

6.1. Glutamate

Glutamate is an excitatory neurotransmitter employed by corticostriatal, thalamostriatal, and thalamocortical projections. It is also the neurotransmitter employed by the STN; therefore glutamate is not only the major driving input to the basal ganglia but also participates in the intrinsic basal ganglia circuits. Glutamate transmission is modulated by dopamine, acetylcholine, GABA, and nitric oxide. Glutamate has been recently related with the pathogenesis of Parkinson's disease. Studies in rats have demonstrated an increase of concentration and release of glutamate from corticostriatal terminals in the striatum after nigrostriatal denervation. Accordingly, studies using glutamate receptor antagonists have shown that they can promote motor behavior and intensify the effect of levodopa.

6.2. Gamma-Aminobutyric Acid

GABA is an inhibitory neurotransmitter used by all the basal ganglia nuclei with the exception of the STN. It is used by the medium spiny neurons to project to the other basal ganglia nuclei. At the same time, the activity of medium spiny neurons is also regulated by GABAergic inputs from striatal interneurons. Moreover, GABA is the neurotransmitter used by the output nuclei to project outside the basal ganglia. GPi/SNr neurons fire tonically at rest suppressing thalamocortical projections. Among all the cortical inputs that basal ganglia receive, they select the desired and appropriate input by suppressing the competing and unwanted programs.

6.3. Dopamine

The dopaminergic system innervates all basal ganglia nuclei and probably exerts powerful modulatory control of the basal ganglia intrinsic circuits. It arises from three main groups of neurons designated

as areas A8 (retrobulbar area, RRA), A9 (SNC), and A10 (VTA). According to connectivity and morphologic features, midbrain neurons are divided in a ventral and a dorsal tier. The dorsal tier includes the dorsal SNC and VTA, and the RRA. Neurons in the dorsal tier are calbindin-positive and innervate the ventral striatum and limbic and cortical areas, as well as the dorsal striatum. The ventral tier is located in the ventral part of the SN and VTA and projects to the striatum. Cells in the ventral tier are calbindin-negative and can be divided in a densocellular part and columns of dopaminergic neurons that penetrate deeply into the SNr. This last group seems to be the first to degenerate in Parkinson's disease.

The dopaminergic system is separated into three different projection systems: the nigrostriatal, mesolimbic, and mesocortical systems. Although the projections of these systems are clearly separated, their neurons of origin in the SN and VTA intermingle.

The nigrostriatal system arises from the SNC, VTA, and RRA and projects to the sensorimotor striatum. The mesolimbic and mesocortical systems originate in the VTA and in the dorsal tier of the SN and RRA. The mesolimbic system projects onto the limbic striatum, the amygdala, and the hippocampus, and the mesocortical system projects onto prefrontal and associative cortices.

There are five types of dopamine receptors that can be classified in two main groups: D1-like and D2-like. D1-like receptors include D1 and D5 receptors and stimulate adenylyl cyclase. D2-like receptors include D2, D3, and D4 receptors and inhibit adenylyl cyclase. They are heterogeneously distributed along the striatum and other basal ganglia nuclei.

The *nigrostriatal dopaminergic projections* are characterized by their convergence with cortical terminals on individual dendritic spine of the spiny neurons, which suggests that one of the main functions of dopamine is to regulate corticostriatal projections. Dopamine also modulates striatal efferents by facilitating the direct pathway via D1 receptors and inhibiting the indirect pathway via D2 receptors. Therefore, the net effect of dopamine in basal ganglia is the facilitation of thalamocortical projections. Activation of dopamine receptors influences neuroplasticity at corticostriatal synapses. Dopamine seems to be necessary not only for maintaining and modulating neuroplasticity but also for inducing it (long-term potentiation and long-term depression). Dopamine also induces plasticity exerted by other neurotransmitters (acetylcholine, nitric oxide, endogenous cannabinoids).

The action of dopamine is more widespread; dopaminergic neurons innervate the GP and the STN and probably regulate intrinsic circuits of the basal ganglia. Interestingly, dopaminergic neurons regulate not only the dopaminergic neurons themselves but also the release of GABA within the SNr and therefore the output projections of the basal ganglia.

The *mesolimbic system* is involved in reward. Dopamine neurons are activated by rewards and reward-predicting stimuli. Aversive stimuli however produce slower dopamine responses that consist predominately of depression. The striatum, frontal cortex, and amygdala also process specific reward information but do not participate in the prediction of reward.

6.4. Acetylcholine

The cholinergic system is of crucial importance in determining the final output from the striatum to other basal ganglia nuclei. Although only around 1% to 2% of the striatal neurons are cholinergic interneurons, the striatum contains the highest concentration of all the cholinergic markers in the brain. Other source of acetylcholine to the striatum comes from the PPN. Cholinergic neurons receive glutamatergic and dopaminergic inputs from the cortex and the SNC, respectively. Although they are sparse, their dendritic trees arborize profusely projecting to the spiny neurons. Spiny neurons receive extensively glutamatergic projections from the cortex. The fact that cortical glutamatergic and striatal cholinergic inputs converge at the level of striatal projection neurons supports the idea that Ach influences striatal function by modulating the corticostriatal glutamatergic transmission.

Striatal cholinergic neurons act over two main subtypes of muscarinic receptors that have opposing effects: M1 receptors, which activate spiny neurons, and M2 receptors with an inhibitory effect. The overall effect of the Ach in the striatum might be the long-term potentiation of the glutamate activation of striatal projection neurons.

The classic view that balance between dopamine and Ach is necessary for normal motor control and that the imbalance of both systems is responsible for parkinsonian motor symptoms has been recently modified, and the current evidence is that the main adaptive response to loss of striatal dopaminergic afferents is, in fact, the hyperactivity of corticostriatal glutamatergic neurotransmission. The benefits observed in Parkinson's disease with anticholinergic drugs might be explained by interaction with

glutamate-mediated transmission. There is also evidence that striatal cholinergic neurons participate in reward-related learning although this process seems to be dopamine dependent.

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Disorders of the Basal Ganglia

Gregory Cooper, Gerald Eichhorn and Robert Rodnitzky

The classic clinical syndromes that result from abnormalities of the basal ganglia are disorders of movement. These may take the form of excessive involuntary movements (i.e., hyperkinesia) or decreased movement (e.g., hypokinesia). Hypokinesias such as bradykinesia (e.g., slow movement) or akinesia (e.g., absence or difficult initiation of movement) are often seen in Parkinson's disease and a few conditions that mimic this disorder. There are several forms of hyperkinesias and many different disease states that cause these symptoms. Among the most common forms of hyperkinesia are chorea, dystonia, tremor, and tics. The complex anatomic connections and physiologic associations of the basal ganglia often make it difficult to determine the anatomic locus of disease responsible for these abnormal patterns of movement. Similarly, with the exception of Parkinson's disease, the neurotransmitter aberrations responsible for many movement disorders are unknown and are often defined only by the mode of action of the drugs used to treat them.

CHOREA

Chorea is an involuntary movement with a variety of causes. It is characterized by arrhythmic, rapid, involuntary movement that flows from one part of the body to another in nonstereotypic fashion. When chorea is severe, of high amplitude, and prominent in proximal parts of an extremity, it is referred to as *ballism*. There are many causes of chorea, including rheumatic fever, metabolic imbalance (e.g., hyperthyroidism), and the use of certain drugs (e.g., amphetamines, levodopa). The form associated with rheumatic fever is known as Sydenham's chorea. One of the most common non-drug-related causes of

chorea is Huntington's disease, an autosomal dominant condition that invariably progresses to severe disability and death.

It has long been known that examination of the brains of patients with Huntington's disease at autopsy reveals remarkable gross atrophy of the head of the caudate nucleus. Modern neuroimaging techniques such as computed tomography or magnetic resonance imaging can demonstrate this finding during the patient's life. At the cellular level, the chorea of Huntington's disease has been related to a loss of striatal GABA-enkephalin neurons that are the origin of the indirect inhibitory pathway to the external segment of the globus pallidus (GPe). It is theorized that reduced inhibition of these pallidal cells leads to increased inhibition of the subthalamic nucleus (STN) by the GPe, in turn reducing the excitatory drive on neurons in the internal segment of the globus pallidus (GPi) from the STN. Decreased inhibitory output from the GPi to the thalamus then results in increased cortical activation and chorea.

This explanation of the origin of chorea in Huntington's disease is consistent with the fact that the most severe and acute form of chorea, ballism, is usually associated with a direct lesion of the STN. The most common of these direct lesions is an infarction of the subthalamic nucleus, resulting in contralateral ballism or hemiballism.

In the chorea of Huntington's disease and the ballism of STN infarction, dopamine-blocking (e.g., haloperidol) or dopamine-depleting (e.g., reserpine) drugs are effective in reducing the abnormal movements, and dopaminergic drugs worsen them. Although dopaminergic cells are not the focus of pathology in either of these conditions, dopamine blockade is effective in reversing the chorea

associated with them. These drugs, by reducing the inhibitory effect of dopamine on striatal GABA-enkephalin neurons, ultimately lead to less downstream inhibition of the subthalamic nucleus (STN). The often remarkable effect of antidopaminergic drugs on chorea is a prime example of the fact that the researcher cannot always infer from a salutary pharmacologic response which cell group or neurotransmitter is primarily affected by the disease process in basal ganglia disorders. One obvious exception to this observation is Parkinson's disease, for which replenishment of a deficient neurotransmitter, dopamine, does reflect the primary neurochemical abnormality.

DYSTONIA

Dystonia, an abnormal hyperkinetic movement that is distinct from chorea, is an involuntary movement that is twisting, somewhat sustained, and often repetitive. With time, the body part affected by this abnormal movement may develop a fixed, abnormal posture.

Dystonia can be described according to its distribution within the body as focal, segmental, or generalized. Focal dystonia implies involvement of a single part of the body such as the hand, whereas segmental dystonia involves two or more adjacent areas of the body, such as the neck and arm. Generalized dystonia is defined as involvement of the lower extremities plus any other body part. Examples of focal dystonia are writer's cramp, an involuntary contraction of hand or finger muscles that occurs while writing, and torticollis, an involuntary turning or tilting of the head. Torticollis plus facial or eyelid dystonia constitute segmental dystonia.

Idiopathic torsion dystonia is the most common condition that causes generalized dystonia. It is an autosomal dominant disorder with variable penetrance that typically begins in childhood or adolescence. The gene for this condition, torsin A, is on the long arm of chromosome 9 at the DYT1 locus. The function of the resultant protein is currently unknown. In this disorder, like most spontaneously occurring dystonic disorders, no apparent structural abnormalities of the basal ganglia and no abnormalities of neurotransmitter function have been consistently demonstrated. However, neurotransmitter manipulation sometimes results in marked reduction of dystonia. The most effective results are obtained with anticholinergic agents. A smaller subset of patients responds remarkably to the dopamine

precursor L-dopa. This dopa-responsive dystonia is inherited in an autosomal dominant fashion, resulting from a mutation to the gene encoding GTP cyclohydrolase, which is involved in dopamine synthesis.

Although there is no obvious structural pathology of the basal ganglia in the idiopathic dystonias, discrete structural lesions of these structures caused by tumor, trauma, or stroke can result in an identical movement disorder. In these cases, the brain abnormality leading to dystonia is most likely to be located in the putamen, but it can also be found in areas related to the basal ganglia through efferent or afferent pathways, such as the thalamus or cerebral cortex. Typically, dystonia caused by unilateral structural abnormalities of the basal ganglia or their connections is confined to the extremities on the contralateral side of the body and is referred to as *hemidystonia*.

Pending the development of better pharmacologic therapies for dystonia based on a fuller understanding of its etiopathogenesis, the use of botulinum toxin has emerged as one of the most effective treatments for this condition. Partial weakening of the muscles responsible for the dystonic movement can be accomplished by directly injecting them with small amounts of botulinum toxin. This substance works by inhibiting release of acetylcholine from nerve terminals, effectively producing chemical denervation of muscle with resultant weakness and atrophy.

TICS

Tics are sudden, brief, stereotyped movements. The movement is involuntary and complex, such as blinking of the eye or shrugging of the shoulder. Movements such as these are known as motor tics. When the vocal apparatus is involved, a tic may consist of a non-specific vocalization, such as a grunt or a distinct verbalization, including recognizable words or phrases. Tourette's syndrome is a condition in which motor and vocal tics occur. Tourette's syndrome, believed to be a hereditary disorder, begins in childhood and is more common in boys. It is often associated with certain behavioral abnormalities such as attention deficit disorder or a variety of compulsions or ritualistic behaviors. Interestingly, first-degree relatives are at a substantially higher risk for Tourette's syndrome, simple motor tics, and obsessive-compulsive disorder (OCD). Like idiopathic torsion dystonia, its anatomic and neurochemical bases are not fully understood. However, the results of structural and functional imaging studies have suggested impaired function of the

basal ganglia. A failure to inhibit subsets of the cortico-striato-thalamocortical circuits has been hypothesized to result in the symptoms of both Tourette's syndrome and OCD. Tics, like chorea, are effectively suppressed by dopamine-blocking drugs, but no definite abnormalities of dopaminergic cells or pathways have been demonstrated in the brains of affected persons.

BASAL GANGLIA DISORDERS CAUSING MULTIPLE FORMS OF ABNORMAL MOVEMENT

Some basal ganglia disorders can result in several different forms of abnormal movement. Several distinct diseases of the basal ganglia can result in several or all of the previously described abnormal movements. Neurodegeneration with brain iron accumulation (NBIA), formerly Hallervorden-Spatz syndrome, a condition associated with neuronal loss and increased iron storage primarily in the substantia nigra pars reticulata (SNr) and internal globus pallidus (GPi), can result in chorea, bradykinesia, or dystonia in any combination. This same spectrum of disordered movements plus tremor can occur in Wilson's disease, a condition characterized by neuronal loss and excess-

sive copper storage in the brain, especially the putamen. In this condition, symptoms can be reversed by decoppering the central nervous system (CNS) with medications.

Tardive dyskinesia is a syndrome in which abnormal movements occur after prolonged administration of dopamine-blocking drugs such as chlorpromazine or metoclopramide. In this condition, the abnormal movement is usually chorea but occasionally is dystonia. The abnormal movements of tardive dyskinesia can affect any part of the body, but they are most often focused on the mouth and tongue. A common but unproved explanation for the pathogenesis of this condition is that dopamine receptors develop compensatory supersensitivity as a result of being subject to chronic pharmacologic blockade.

The class of dopamine receptor that is blocked seems to be important in the pathogenesis of this syndrome, because dopamine-blocking drugs that preferentially block the D₃ rather than the D₂ dopamine receptor are much less likely to cause tardive dyskinesia.

SELECTED READINGS

DeLong MR, Wichmann T. Circuits and circuit disorders of the basal ganglia. *Arch Neurol.* 2007;64(1):20–4.

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1. INTRODUCTION

The thalamus is the largest structure of the mammalian diencephalon. It comprises many nuclear groups, each concerned with transmitting characteristic afferent signals to specific areas of the cerebral cortex. The thalamus is often described as the gateway to the cerebral cortex. The term *thalamus* is a Greek word meaning “inner chamber.” Its origin dates back to the 2nd century AD, when Galen traced the optic-nerve fibers to an oval mass closely associated with the ventricles. This part of the brain, known as the optic thalamus, was later defined as a large mass of gray matter involved with visual stimuli and in the processing of all sensory modalities except olfaction. The size of the thalamus is relatively small compared with that of the neocortex, but the functions of each major neocortical area largely depend on the interactions with well-defined thalamic cell groups. For this reason, an increase in size of any neocortical area is correlated with a

corresponding increase in the related thalamic nuclei. The nomenclature of the different nuclear subdivisions of the primate thalamus is more complex than in rodents. Although the thalamic cytoarchitecture in monkeys and humans is relatively similar, the nomenclature used to define thalamic nuclei in simians and humans has diverged so much over the years that nonspecialist readers surely believe that the thalami of humans and monkeys are fundamentally different. The main difference between the human and non-human primate thalamus is the relative growth of specific thalamic nuclei relative to other nuclear subdivisions; the pulvinar is the most representative example of a thalamic nucleus that has overgrown in the human brain.

This chapter provides an overview of the main features that characterize the anatomy, chemistry, and functional organization of the primate thalamus. For additional information, the reader is referred to other comprehensive reviews and compendiums (Steriade and Deschenes, 1984; Steriade and McCarley, 1990; Sherman and Koch, 1998; Kultas-Ilinsky and Ilinsky, 2001; Sherman and Guillory, 2001; Pinault, 2004; Jones, 2007).

2. HISTORY

Thomas Willis first introduced the thalamus to the Western medical literature through his writings in 1664 and 1681 in which he refers to a thalamus *nerorum opticorum*. He noticed the large size of the diencephalon compared with the cerebral hemispheres in bird and fish. The first report of clear nuclear subdivisions in the thalamus dates back from the beginning of the 1800's when Karl Friedrich Burdach identified the internal medullary lamina and divided the thalamus into superior, external, and internal nuclei. He was then followed by Luys in 1865, who identified four thalamic centers, namely the centre anterieur, centre moyen, centre median, and centre postérieur. Through brain dissections, he noticed that these centers were connected through white matter tracts to the cerebral cortex. In 1872, Meynert divided the lateral thalamic nucleus into a dorsal, lightly myelinated, and a ventral, heavily myelinated, region. He also noticed that different regions of the thalamus were connected with different cerebral lobes through massive fiber peduncles called thalamic peduncles. He provided the first description of the habenular nuclei and the habenulo-interpeduncular fasciculus (also called fasciculus retroflexus). Further nuclear subdivisions of the thalamus in various species were then refined and published in numerous assays in the late 1800s.

In 1891, Edinger first described the classic thalamic pain syndrome, but the precise etiology was first recognized by Dejerine and Egger in 1903. Some of the best modern descriptive accounts of the thalamus appeared in the early 1900s from the laboratory of Cecile and Oskar Vogt (1909), but their nomenclature was rather complex and cumbersome, though many terms introduced by these scientists retained their popularity. Santiago Ramon y Cajal used Golgi- and Nissl-stained sections to identify many nuclei in the rabbit thalamus, which were grouped into three groups called the external, middle, and anterior series. One of Cajal's main contributions to the cellular organization of the thalamus was the clear distinction between interneurons and relay neurons. In 1932, Le Gros Clark published a detailed review article in which he surveyed much of the previous literature and described his preliminary data on the thalamocortical systems in rats. The culminating point of this period was the publication of Walker's influential book *The Human Thalamus* in 1938. The next important contribution to the thalamus anatomy came from Rose and Woolsey who used retrograde

degeneration methods to demonstrate that thalamic nuclei were connected with specific areas of the cerebral cortex. They also considered the possibility that neurons in a specific thalamic nucleus could have axon collaterals to more than one cortical region.

The era of modern thalamic physiology started in the 1940s. Some of the key advancements in this field include the studies of Marshall in 1941, who showed that sensory inputs from the periphery increases activity of a specific group of cells in the ventroposterior thalamic nucleus. This work led to the development of studies of sensory transmission in the thalamus by means of evoked potential recordings, later followed by single unit studies. Another important historical step in our understanding of modern thalamic physiology came from the discovery of specific and nonspecific thalamocortical pathways. The first evidence for nonspecific thalamocortical connections came from the seminal studies of Morison and Dempsey who could evoke two types of evoked responses, so-called primary and recruiting responses, in the cerebral cortex of anesthetized cats after stimulation of relay or intralaminar thalamic nuclei, respectively. The existence of a diffuse thalamocortical system that arises from intralaminar nuclei was later confirmed and studied in further detail by Jasper and his colleagues who showed that recruiting responses induced by intralaminar thalamic stimulation were maintained after selective ablation of relay nuclei. A few years later came the studies of Moruzzi and Magoun (1949), who provided evidence for the so-called reticular activating system considered as the main substrate for desynchronized EEG cortical activity in state of arousal. Stimulation of the midbrain was considered most effective in eliciting these responses, which were thought to be mediated by diffuse thalamocortical projections from intralaminar nuclei, thereby considered as a rostral extension of the reticular formation. In the 1960s, the application of intracellular microelectrode techniques combined with the development of more sophisticated anatomic tracing methods opened up the field for a huge amount of new discoveries about thalamic physiology and connectivity. The analysis of thalamic oscillations in relation to behavioral and pathologic states has had a major impact in our current view of thalamic functions. Finally, the recent development of sophisticated patch clamp recording method, single cell tracing studies, immunocytochemical techniques, and powerful molecular biology approaches have resulted in an explosion of new discoveries that highlight the complex features of thalamic organization and further demonstrate its

complex integrative properties and function in normal and pathologic brain activities (see Jones [2007] for more information).

3. NUCLEAR SUBDIVISIONS OF THE THALAMUS

The dorsal portion of the diencephalon comprises three major parts: the epithalamus, the dorsal thalamus, and the ventral thalamus (Fig. 1). The *epithalamus* is an important component of the limbic system that consists of the pineal body, the habenular nuclei, the stria medullaris, and the associated paraventricular nuclei. The relationships of these nuclei with basal ganglia structures and their potential role in limbic functions will be discussed later in this chapter. The pineal body will not be discussed further in this chapter because of its limited connection with the thalamus. The *dorsal thalamus* is divided into anterior, medial, ventrolateral, and posterior nuclear groups by a band of myelinated fibers, the internal medullary lamina of the thalamus (Fig. 1). The *anterior nuclear group* forms a rostral swelling that protrudes from the dorsal surface of the rostral thalamus. It is separated from other thalamic nuclei by a myelinated capsule. The *lateral nuclear group* comprises two main nuclear masses, and the ventral nuclear mass, which extends throughout the entire rostrocaudal extent of the thalamus, is divisible into separate nuclei: (1) the ventral posterior nucleus, medial geniculate nucleus, and dorsal lateral geniculate nucleus caudally; (2) the ventral lateral nucleus at intermediate levels; and (3) the ventral anterior nucleus rostrally (Fig. 1). These are the ventral-tier thalamic nuclei. The lateral nuclear mass located dorsal to the ventral nuclear mass also comprises three major nuclei: (1) the pulvinar, which occupies a large part of the caudal thalamus; (2) the lateral posterior nucleus, located at an intermediate level; and (3) the lateral dorsal nucleus, the most rostral component of this nuclear mass. These are known as the dorsal-tier thalamic nuclei. The *medial nuclear group*, located medial to the internal medullary lamina, is largely made up of the mediodorsal nucleus, a major relay center for cognitive information to the prefrontal cortex. A fourth thalamic nuclear group, confined within the boundaries of the internal medullary lamina, is the *intralaminar nuclei*. These nuclei, which are often considered as nonspecific or diffusely projecting nuclei, are divided into two main groups: the anterior group, which contains the paracentral and centrolateral nuclei, and the posterior group represented by the

centromedian and parafascicular nuclei. The so-called *midline thalamic nuclei* are more or less distinct cell clusters along the medial portion of the thalamus. These nuclei are smaller and more difficult to delimit in humans and monkeys than in rodents. The main cell groups are the paraventricular, rhomboid, median central, and reuniens nuclei (Fig. 1). These nuclei are directly connected with cortical and subcortical limbic-related brain regions. Along the lateral border of the thalamus, near the internal capsule, lies the external medullary lamina, which separates the *reticular nucleus* from the remainder of the thalamus. The reticular nucleus forms a thin outer envelope that tightly surrounds the entire extent of the dorsal thalamus. In contrast with all other thalamic nuclei that project to the cerebral cortex, the reticular nucleus does not provide cortical inputs but instead innervates other thalamic nuclei and the brain stem. The *ventral thalamus* comprises the ventral lateral geniculate nucleus, the zona incerta, and the fields of Forel (Fig. 1).

In general, most thalamic nuclei can be classified either as modality-specific nuclei, multimodal-association nuclei, or nonspecific, diffusely projecting nuclei (Fig. 1). The modality-specific nuclei are reciprocally connected with well-defined cortical areas that are related to specific motor or sensory functions. However, the multimodal-association nuclei have widespread cortical connections, with association areas in the frontal, parietal, and temporal lobes. Unlike modality-specific nuclei, they do not receive inputs from one dominant subcortical structure but are rather innervated by many different afferent inputs that have equal weight. Consistent with such a pattern of innervation, the functions of association nuclei are not precise and modality-specific but are related to higher functions such as language and learning. Finally, the nonspecific or diffusely projecting nuclei comprise the intralaminar and midline thalamic nuclei that provide widespread cortical projections and innervate the striatum more massively than the cerebral cortex (Fig. 1).

The human thalamus is strikingly larger than in any other species. It is twice as large as that of great apes and at least five times larger than that of old world monkeys. The pulvinar, the center median/parafascicular complex, and the ventrolateral nuclear group are most particularly enlarged in humans. Despite this significant expansion, the nuclear subdivision of the human thalamus is very similar to that in monkeys. However, because the nomenclatures used to identify monkey and human thalamic nuclei have

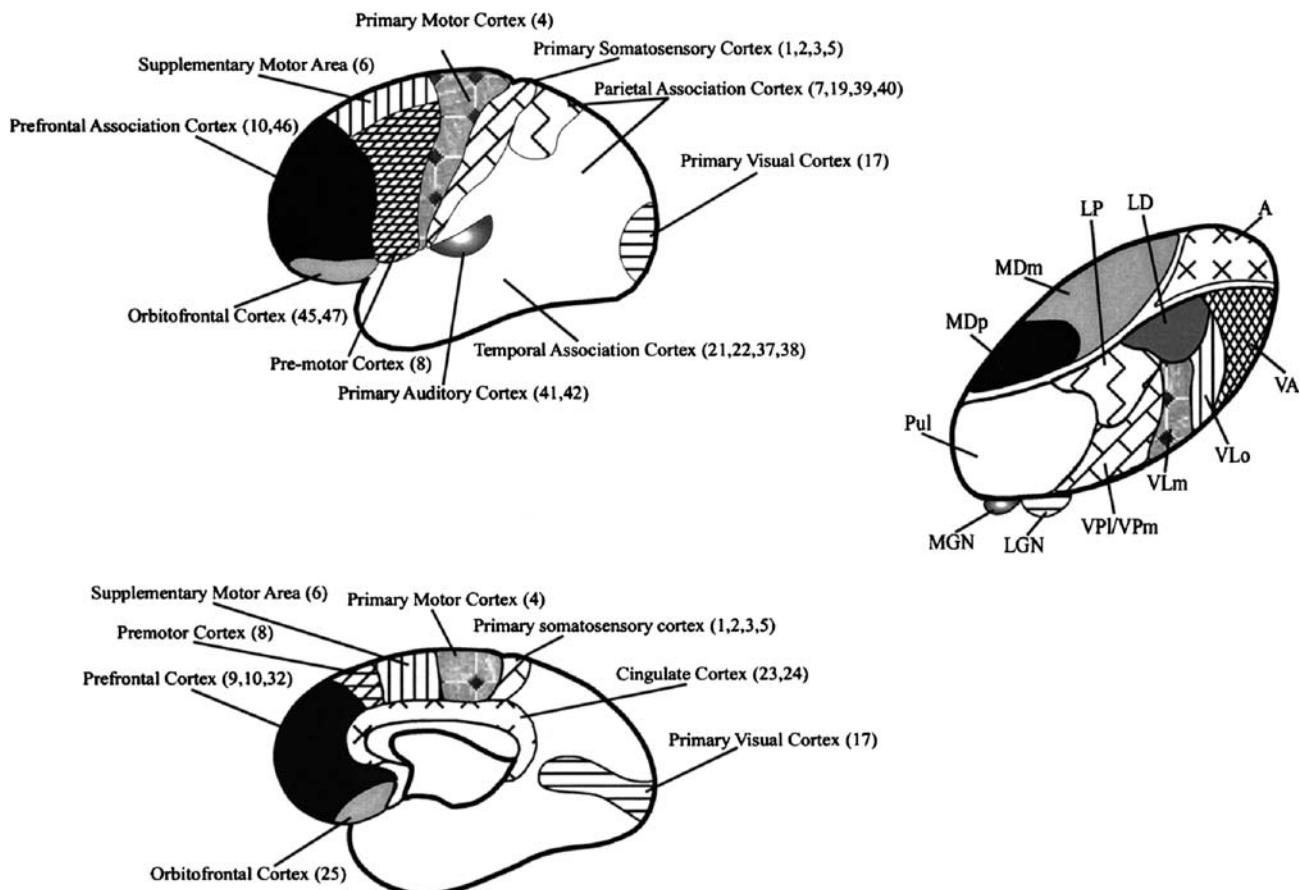


Fig. 1. Thalamocortical connections of modality-specific and association thalamic nuclei. The Brodmann's cortical areas are indicated in parentheses. A, anterior thalamic nuclei; LD, laterodorsal nucleus; LGN, lateral geniculate nucleus; LP, lateral posterior nucleus; MDM, mediiodorsal nucleus, magnocellular part; MDp, mediiodorsal nucleus, parvocellular part; MGN, medial geniculate nucleus; Pul, pulvinar; VA, ventral anterior nucleus; VLm, ventrolateral nucleus, pars medialis; VLo, ventrolateral nucleus, pars oralis; VPI, ventroposterolateral nucleus; VPM, ventroposteromedial nucleus.

diverged so much over time, it is very difficult for “nonexperts” to recognize corresponding nuclear regions between humans and non-human primate thalamus in the modern literature. Although various attempts have been made to reach an agreement, there is still serious discrepancy between human and monkey thalamus nomenclature. Dialogues between basic and clinical scientists would benefit tremendously from the use of a common thalamic nomenclature between human and nonhuman primates (see Jones [2007] for review).

4. CELL TYPES OF THE THALAMUS

The mammalian thalamus comprises three major cell types: (1) the relay cells, which project their axons to the cerebral cortex or the striatum; (2) the interneurons, which are not present in all nuclei in

non-primates and have their axons and synaptic connections confined within the nuclei in which they lie; and (3) the reticular neurons, which have their perikarya confined within the limits of the reticular nucleus and send their axons to the dorsal thalamus (Fig. 2). Relay neurons are glutamatergic, whereas both interneurons and reticular neurons use gamma-aminobutyric acid (GABA) as a neurotransmitter. The morphology of these three cell types is strikingly different. In most thalamic nuclei, relay cells have a bushy appearance, containing a relatively symmetrical dendritic field with occasional dendritic appendages or protrusions, but with no apparent morphologic characteristics that correspond with their physiologic properties. In the lateral geniculate nucleus (LGN), relay cells in the parvocellular laminae have smaller somata and bitufted dendrites confined to the lamina in which they lie, whereas magnocellular neurons have larger

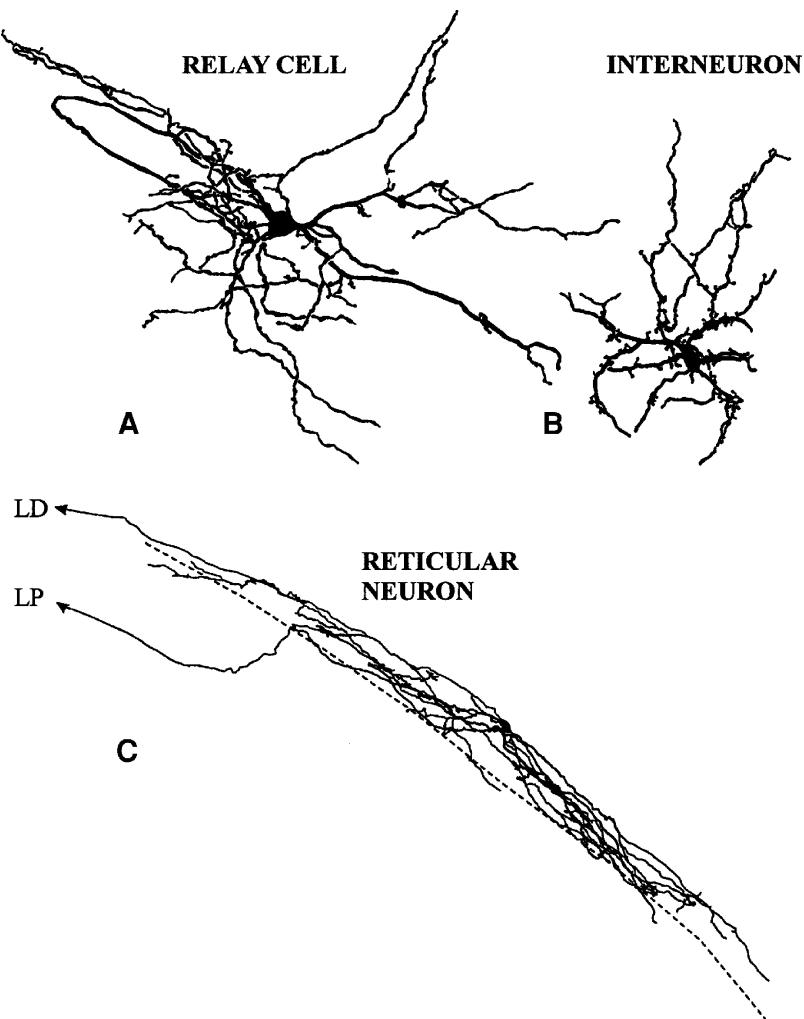


Fig. 2. The three major cell types of the mammalian thalamus drawn from (A, B) Golgi preparation or (C) intracellular filling. (A and B from Jones E. *The Thalamus*. New York: Plenum Press, 1985, with permission.)

somata and more radial dendritic fields that cross laminar boundaries. Overall, relay cells have larger somata than those of interneurons, but they exhibit a broad range of sizes. For instance, the perikarya of relay cells in the ventral posterior and ventral lateral nuclei of monkeys range from $70 \mu\text{m}^2$ to more than $400 \mu\text{m}^2$. In the LGN of monkeys, magnocellular neurons have somata that range from 25 to 40 μm in size, whereas cells in the parvocellular laminae range from 16 to 25 μm in diameter. However, cells in the S and interlaminar zones have 8- to 10- μm soma.

The GABAergic interneurons, which are found in all thalamic nuclei in primates, are much smaller than relay cells. In general, their somata are less than 10 μm in diameter, with a few short dendrites that give off numerous lengthy processes that end in terminal boutons and form dendro-dendritic synapses

(Fig. 2). Interneurons form approximately 30% of the total neuronal population in all thalamic nuclei, except in intralaminar nuclei, where they are slightly less abundant and account for about 15% to 20% of neurons. It is noteworthy that dorsal thalamic nuclei in rodents, except the lateral geniculate nucleus, are practically devoid of GABAergic interneurons. Hence, rodents have a unique thalamus in which processing of extrinsic information and thalamocortical outflow are under the sole influence of GABAergic inputs from the reticular nucleus and basal ganglia (see later).

The cells of the reticular nucleus are relatively large, with somal diameters ranging from 25 to 50 μm in monkeys and humans (Fig. 2). The cells are flattened so that the dendritic field is commonly discoidal, and they densely overlap at all levels of the nucleus.

Although it has long been considered as a homogeneous nucleus, recent single-cell filling studies have revealed a significant structural heterogeneity. Three types of reticular neurons have been identified in rats based on three-dimensional reconstruction of their dendritic tree: (1) cells with dorso-ventral dendritic ramifications, (2) cells with rostro-caudal dendritic ramifications and (3) cells with dendritic ramifications in all directions. The axons of reticular neurons have short intranuclear collaterals, and in some species, the dendritic branches form dendro-dendritic synapses. All reticular cells are GABAergic and project to all dorsal thalamic nuclei. Structural and morphometric analyses of the axonal projections from juxtacellularly stained reticular neurons in rats revealed complex and variable patterns of distribution within dorsal thalamic nuclei. Based on these anatomic observations, two types of regulatory processes of reticular projections over thalamocortical neurons have been proposed: (1) ordered reticulothalamic axonal projections toward first-order and high-order thalamic nuclei and (2) divergent reticulothalamic projections toward at least two separate thalamic nuclei. Based on their pattern of connectivity, thalamocortical and reticular neurons form open- and closed-loop connections, which likely represent anatomic substrates of mechanisms for lateral and feedback inhibitions, respectively. Although it has long been thought that the reticular nucleus does not project to the anterior thalamus, several anatomic studies have now demonstrated reticular inputs to these nuclei in rats, cats, and monkeys. Apart from GABA, other chemicals found in reticular neurons include calcium binding proteins (parvalbumin, calretinin, and calbindin), somatostatin, thyrotropin-releasing hormone, vasoactive intestinal peptide, neuropeptide Y, and prolactin-releasing peptide (see Pinault, 2004).

Both corticothalamic and thalamocortical neurons give off axon collaterals to very specific sectors of the reticular nucleus. The topographic arrangement of these projections makes different sectors of the reticular nucleus specifically dedicated to a particular dorsal thalamic relay nucleus. The orientation of the dendritic arbors of reticular neurons match the organization of the thalamocortical and corticothalamic inputs that converge upon segregated functional sectors of the nucleus. However, most dendrites usually span more than one functional tier of the nucleus, implying that single neurons may receive inputs from functionally divergent thalamic or cortical areas. In line with this, reticular neurons are not modality-specific and have larger receptive fields than cells in

the somatosensory cortex and the ventroposterolateral thalamic nucleus. Projections to and from intralaminar and midline nuclei are more diffuse and less topographic. The reticular nucleus also receives dense brain-stem inputs from cholinergic noradrenergic and serotonin cell groups. These modulatory afferents control the firing rate and pattern of reticular neurons in different states of vigilance and influence all thalamocortical activity. Basal forebrain cholinergic and GABAergic inputs from the basal nucleus of Meynert, substantia innominata, and globus pallidus have also been reported (see Steriade and Deschenes [1984] and Pinault [2004] for more information).

5. BASIC SYNAPTIC ORGANIZATION OF THE DORSAL THALAMUS

In most relay and intralaminar thalamic nuclei, four types of synaptic terminals are found and named according to the size and the shape of vesicles they contain. They are termed: (1) RL, round vesicles and large size; (2) RS, round vesicles and small size; (3) F, flat vesicles; and (4) PSD, presynaptic dendrites (Fig. 3). The RL terminals are relatively abundant,

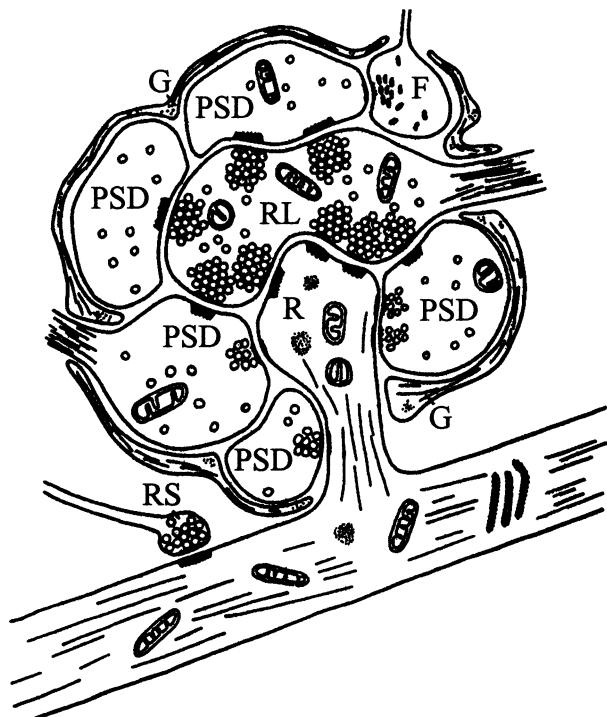


Fig. 3. Complex glomerular synaptic arrangement of major synaptic inputs to thalamic relay neurons. G, glial processes; R, relay neuron; RL, round vesicles and large size; RS, round vesicles and small size; F, flat vesicles; PSD, presynaptic dendrites.

are packed with small synaptic vesicles, usually contain many mitochondria, and form asymmetric synapses. They arise mainly from the main extrinsic afferents to relay nuclei, which include the medial lemniscus, cerebellum, optic tract, mammillothalamic tract, and inferior colliculus. The RS terminals, which are much more numerous than RL terminals, also contain small, round vesicles and form asymmetric synapses mostly with distal dendrites of thalamocortical neurons. They largely originate from layer VI of the cerebral cortex. The F terminals are GABAergic, contain flattened synaptic vesicles, and form symmetric synapses. The reticular nucleus and axons of interneurons, when present, are the main sources of these terminals. The PSD terminals contain aggregates of pleomorphic synaptic vesicles, significant quantities of rough endoplasmic reticulum (ER), and clusters of free ribosomes. They arise from dendrites of GABAergic interneurons. In general, the RL and PSD terminals form synapses with the proximal part of relay neurons, whereas RS boutons mostly terminate on distal dendrites of relay neurons, although a substantial proportion also innervates dendrites of interneurons. F-terminals end on the proximal part of relay neurons and interneurons as well as on the PSD of interneurons. Detailed quantitative measurements of the relative abundance of these different types of terminals on specific neuronal populations in cats and monkeys have been performed. Overall, the pattern of distribution of afferent terminals on thalamic neurons is relatively similar across primates and non-primates (e.g., subcortical afferents are concentrated on the proximal dendrites and cell bodies, whereas corticothalamic terminals are evenly distributed across the somatodendritic domain). The degree of convergence of cortical and subcortical afferents from various sources onto individual thalamocortical neurons varies between thalamic nuclei. For instance, in the LGN, each relay neuron is innervated by a single type of retinal ganglion-cell axon, whereas in the ventroposterior nucleus, indirect evidence suggests that spinothalamic and medial lemniscus afferents partly converge on the same neurons. It is very likely that RS terminals that end onto individual thalamocortical neurons arise from cortical layer VI neurons. There is also evidence that a subpopulation of corticothalamic terminals arise from layer V neurons (see later). These terminals display the ultrastructural features of RL boutons and form asymmetric synapses with dendrites of relay neurons and PSD of interneurons. The F terminals that arise from the reticular nucleus innervate the full length of relay-cell dendrites, whereas

F terminals from the basal ganglia output structures, the substantia nigra pars reticulata (SNr) and the internal globus pallidus (GPi), are largely confined to the proximal part of relay neurons in the ventral anterior and ventral lateral nuclei. In general, interneurons receive much less innervation from RL and RS terminals than do relay cells. For instance, less than 10% of cerebellar RL terminals end on PSD in the monkey ventrolateral nucleus. Axons from single cerebellar neurons form 3 to 4 times more synapses with dendrites of relay neurons than with interneurons. Despite this relatively light innervation, electrophysiologic data clearly demonstrate that stimulation of either the cerebral cortex or subcortical glutamatergic afferents induces disynaptic inhibitory postsynaptic potentials (IPSPs) in relay neurons, indicating the effectiveness and strength of these connections. Similarly, IPSPs were recorded in relay neurons of the anterior nuclei after stimulation of the mammillothalamic tract in cats.

6. SYNAPTIC CONNECTIVITY OF THE RETICULAR NUCLEUS

As mentioned previously, the reticular nucleus does not project to the cerebral cortex, but rather provides GABAergic inputs to most thalamic relay nuclei and the brain stem. In turn, reticular neurons receive glutamatergic inputs from axon collaterals of corticothalamic and thalamocortical neurons as well as modulatory cholinergic and monoaminergic inputs from the tegmental pedunculopontine nucleus, nucleus raphe, and locus coeruleus. In addition, neurons in the basal forebrain and globus pallidus as well as axon collaterals and/or PSD of reticular neurons provide substantial GABAergic inputs to reticular neurons. There is considerable evidence that the ascending brain-stem inputs, acting both on the reticular nucleus and other thalamic nuclei, control the functional state of the thalamus as well as the transition from wakefulness to sleep. Reticular neurons have complex firing patterns that depend on the subject's state of vigilance. During deep sleep, thalamic neurons fire in rhythmic bursts that increase hyperpolarization of thalamocortical neurons, thereby inhibiting transfer of sensorimotor information to the cortex. In an awake state or during paradoxical sleep (REM sleep), the ascending brain-stem projections suppress reticular neuron activity and activate other thalamic nuclei, thereby facilitating the relay of information through the thalamocortical system. Under these conditions, thalamocortical neurons function in a relay-type mode, actively process

arriving subcortical information, and transmit it to the cortex.

The role of corticothalamic inputs and intrinsic GABAergic connections has been the subject of intensive research. Corticothalamic projections facilitate synchronized activity in thalamo-corticothalamic circuits and thereby initiate and maintain thalamic rhythms that underlie changes in the electroencephalogram that accompany changes in conscious states. Stimulation of cortical neurons induces fast monosynaptic excitatory postsynaptic potentials (EPSPs) followed by fast GABA-A-mediated and slow GABA-B-mediated IPSPs in cat reticular neurons. Although the exact functions of these IPSPs has not yet been determined, there is strong evidence that they may play an important role in controlling burst discharges in reticular neurons, thereby influencing oscillations in the thalamic network.

Projections from the reticular nucleus to the dorsal thalamus are topographically organized (e.g., sectors of the reticular nucleus that project to a particular thalamic nucleus receive projections back from this nucleus). Quantitative analysis of the synaptology of Reticular Nucleus (RTN) neurons in rats revealed that 50% of synapses on proximal dendrites derive from corticothalamic axons, 30% to 40% from thalamocortical axons, and 0 to 25% from GABAergic inputs. On distal dendrites, 60% to 65% of inputs arise from the cerebral cortex, 20% are from thalamocortical axons, and about 15% are GABAergic (Jones, 2007).

7. NEUROTRANSMITTERS IN THE THALAMUS

7.1. Glutamate and Related Enzymes

As discussed previously, glutamate and GABA are the two transmitters released at most synapses in the dorsal thalamus. The corticothalamic and major spinal, tegmental, and cerebellar afferents to modality-specific thalamic nuclei use glutamate as neurotransmitter. Thalamic projection neurons are enriched in vesicular glutamate transporter 2 (vGluT2) mRNA. Although a low level of vGluT1 has also been observed in specific thalamic nuclei in rats, human, and monkey, data strongly suggest that vGluT1 and vGluT2 are largely segregated and represent specific markers of cortical and thalamic output neurons, respectively. Overall, the thalamus is enriched in all types of glutamate receptors. Ionotropic, α -Amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), N-methyl-D-aspartate (NMDA), and kainate-receptor subunits, as

well as different subtypes of metabotropic glutamate receptors, are expressed to varying degrees in relay neurons and thalamic interneurons in primates and non-primates. For instance, in the monkey thalamus, there are low levels of gene expression for the AMPA/kainate subunits GluR1, GluR2, GluR5, and GluR7, whereas GluR3, GluR4, and GluR6 subunits are expressed at a moderate level. The five genes (NMDAR1 and NMDAR2A-D) that encode subunits of the NMDA receptors are all found to some extent in the dorsal thalamus. In general, the NMDAR1 and NMDAR2B subunits are expressed at higher levels than are the other NMDAR2 subunits. The mRNAs for the three main groups of metabotropic glutamate receptors (group I, mGluR1, 5; group II, mGluR2, 3; group III, mGluR4–8) have been identified in the rodent and primate thalamus. The mRNAs for mGluR1, mGluR4, and mGluR7 are highly expressed throughout the dorsal thalamus, whereas mGluR3 mRNAs are particularly abundant in the reticular nucleus. Very low levels of mGluR2 mRNAs are found throughout the thalamus.

Glutamatergic thalamic relay neurons are also enriched in α -calcium/calmodulin-dependent protein kinase type II (CAMKII- α), an enzyme specifically found in forebrain glutamatergic neurons. There are four different isoforms of CAMKII ($\alpha, \beta, \gamma, \delta$). The α and β forms are expressed only in neurons, whereas the γ and δ isoforms are found in both nerve cells and cells of other tissue. Although all isoforms are expressed in the thalamus, CAMKII- α is the most abundant across thalamic nuclei. At the subcellular level, CAMKII- α is associated with the postsynaptic densities of glutamatergic synapses on glutamatergic neurons. Except for the monkey dorsal lateral geniculate nucleus where only a subset of cells express CAMKII- α , all other thalamic relay neurons in the dorsal thalamus contain the enzyme mRNA and protein, but to a variable degree depending on the nuclear localization, cell packing density, and size of labeled neurons. The anteroventral, ventral medial, ventral medial geniculate, inferior, and medial pulvinar are the most enriched thalamic structures. Other nuclei have intermediate levels, and neurons in the lateral geniculate nucleus contain the lowest level of expression. Staining is absent from GABAergic interneurons as well as GABAergic cells in the reticular nucleus, zona incerta, and pregeniculate nucleus. Among the other isoforms, CAMKII- β and CAMKII- δ are expressed to a moderate level in specific thalamic nuclei, whereas CAMKII- γ is almost completely absent from the thalamus. For each of the

three isoforms expressed in the thalamus, the caudal intralaminar nuclei, center median, and parafascicular stand out by their relative low expression in CAMKII mRNA.

7.2. GABA

In most thalamic nuclei in primates, GABA is derived from both reticular neurons and local interneurons, but in rodents, reticular neurons are the sole source of intrathalamic GABAergic connectivity. In the ventral and posterior intralaminar nuclear group, basal ganglia afferents from GPi and SNr also provide strong GABAergic influences in both primates and non-primates. Both GABA-A and GABA-B receptors mediate inhibitory transmission throughout the thalamus. At least 10 of the 15 GABA-A receptor subunits have been identified in thalamic nuclei. GABA-A receptor densities, identified by autoradiographic ligand-binding studies, are high throughout the thalamus, except in the reticular nucleus. In fact, the reticular nucleus displays a pattern of GABA-A receptor subunits that differs from that of dorsal thalamic nuclei. Many subunit mRNAs expressed at very high levels in dorsal thalamic nuclei and throughout the brain ($\alpha 1$, $\alpha 2$, $\beta 2$ subunits) are either undetectable or expressed at low levels in the reticular nucleus. GABA-B receptor binding is very dense in both the rat and primate thalamus, which is consistent with electrophysiologic studies showing that GABA-A- and GABA-B-mediated fast and slow IPSPs can be induced in thalamic relay neurons after stimulation of extrinsic inputs or reticular afferents. At the ultrastructural level, GABABR1 and R2 subunits are expressed pre- and postsynaptically in glutamatergic terminals and dendrites of relay neurons, respectively. Postsynaptic receptors are largely extrasynaptic (i.e., at nonsynaptic sites along the dendritic plasma membrane of thalamic neurons). Connections between reticular thalamic neurons elicit their effects throughout both GABA-A and GABA-B receptors.

7.3. Monoaminergic Systems

In addition to glutamate and GABA, modulatory inputs from brain-stem monoaminergic and cholinergic cell groups are needed to modulate the activity of ensembles of thalamocortical neurons, thereby setting the state of brain activity. Serotonergic and noradrenergic terminals and receptors are found, to some extent, in all thalamic nuclei. These brain-stem inputs, which originate from the raphe nucleus and

the locus coeruleus, respectively, arborize profusely throughout most of the thalamus. In monkeys, noradrenergic fibers are most particularly abundant in the dorsal thalamic nuclei near the midline, the mediodorsal nucleus, the posterior-pulvinar and reticular nuclei. At the ultrastructural level, noradrenergic axon terminals are small and, for the most, do not form conventional synapses. Apart from the habenula and reticular nucleus, the noradrenergic $\alpha 1b$ receptor mRNA is strongly expressed in all other thalamic nuclei. In contrast, the $\alpha 1A/D$ is heavily expressed in the reticular nucleus, but almost completely absent from the rest of the thalamus. Norepinephrine application or locus coeruleus stimulation prolongs tonic firing of thalamic relay cells and reticular neurons. This slight depolarization, mediated by alpha 1 receptor activation, inhibits burst firing and promotes single-spike activity, thereby reducing thalamic oscillation. In contrast, blockade of alpha 1 receptors *in vivo* leads to decreased discharge rates in reticular neurons.

The serotonergic afferents to the thalamus arise from the dorsal and median raphe nuclei. Serotonin fibers innervate all thalamic nuclei. In non-human primates, the paraventricular, intralaminar, suprageniculate-limitans, medioventral, reticular, and ventral lateral geniculate nuclei receive the densest serotonergic innervation. As for the noradrenergic inputs, most serotonin-containing terminals are closely apposed to, but do not form clear synaptic contacts with, thalamic neurons. Significant 5HT1a receptor mRNA expression was found in intralaminar nuclei, lateral geniculate nucleus, medial habenula, reticular nucleus, and zona incerta, and high levels of 5HT1C mRNA are expressed in the laterodorsal, lateral posterior, medial geniculate, posterior, habenular, and paraventricular nuclei. The 5HT4 receptor is absent from the thalamus, whereas the 5HT7 is widely expressed, but particularly abundant in the anterior, paraventricular, and midline intralaminar nuclei. Serotonin mediates complex and variable physiologic effects on thalamic neurons depending on the nucleus in which it is released. For instance, serotonin slightly depolarizes neurons in the lateral and medial geniculate nuclei *in vitro*, whereas it hyperpolarizes relay neurons in other dorsal thalamic nuclei. In the reticular nucleus, serotonin causes a profound depolarization and moves the membrane potential of reticular neurons toward the tonic discharge mode. Serotonin excites thalamic interneurons.

A modest histaminergic projection from the tuberomammillary region to the paraventricular, habenular, lateroposterior, as well as lateral and medial geniculate nuclei has been described. The presynaptic H3 receptor is the most abundant thalamic histaminergic receptor, being expressed widely throughout the mammalian thalamus. Albeit to a lower level, postsynaptic H1 and H2 receptors are also present. Like norepinephrine and serotonin, histamine promotes a change from burst to tonic firing and can enhance transmission of sensory signals through the thalamus. It also increases tonic discharges in interneurons. Because hypothalamic histamine neurons are spontaneously active during wakefulness and stop firing in slow wave sleep, they may widely affect thalamic neurons in relation to the state of consciousness.

Until recently, dopamine was not considered as a major transmitter of thalamic afferents. However, recent studies in non-human primates emphasized the existence of this system. Both studies revealed a significant dopaminergic innervation of midline, associative, and ventral motor nuclei in rhesus monkeys. In contrast, the intralaminar and relay sensory nuclei contain the lowest amount of dopamine axons. However, there is some controversy between these studies regarding the source(s) of this innervation. On one hand, some authors reported that it originates mainly from axon collaterals of the nigrostriatal dopaminergic pathway and degenerates in 1-methyl-4-phenyl-1,2,3,5-tetrahydropyridine (MPTP)-treated monkeys, whereas others demonstrated a more diverse origin from various hypothalamic, brain stem, and mesencephalic dopaminergic neuronal groups, with a limited contribution from the SNc. These findings concur with biochemical studies showing the presence of dopamine in the human and monkey thalamus. Moreover, D2-like dopamine receptor binding sites have been shown in the human thalamus with a distribution that resembles that of the dopamine innervation. Strong perikaryal D5 immunolabeling is found throughout the human thalamus. Although much work remains to be done to unravel the functional significance of dopamine at the thalamic level, these anatomic data provide a solid foundation for a robust and complex thalamic dopamine system that likely mediates broad influences on neuronal activity in various cortical and subcortical regions through thalamofugal connections. Its possible degeneration in the monkey model of Parkinson's disease provides evidence for a critical extrastriatal site whereby dopamine depletion could induce significant pathologic changes in neuronal activity and behavior.

7.4. Cholinergic Systems

The thalamus receives dense cholinergic innervation from the brain-stem pedunculopontine and laterodorsal tegmental nuclei. The pulvinar and parts of the lateral geniculate nucleus also receive inputs from the mesencephalic parabigeminal nucleus. A more modest cholinergic innervation of the reticular nucleus arises from basal forebrain neurons in the basal nucleus of Meynert and diagonal band of Broca. The anterior, intralaminar, ventral, geniculate, habenular, and reticular nuclei receive the densest cholinergic input. At the electron microscopic level, cholinergic terminals are medium-to large-sized and mainly form symmetric synapses, though a few instances of asymmetric contracts have also been reported. Both muscarinic and nicotinic cholinergic receptors mediate slow-inhibitory and fast-excitatory effects of acetylcholine in thalamic neurons, respectively. For instance, reticular neurons are slowly inhibited by acetylcholine application, probably through activation of M2 muscarinic receptors, whereas nicotinic receptors mediate fast cholinergic excitation of relay neurons. Of the five muscarinic-receptor subunit mRNAs, M2 and M3 are abundant throughout the dorsal thalamus and reticular nucleus, whereas M1 and M4 are practically absent.

A similar degree of heterogeneity is also found for nicotinic-receptor subunits. The $\alpha 3$, $\alpha 4$, and $\beta 2$ nicotinic receptor subunits are widely expressed throughout the thalamus, but the $\alpha 4$ displays a heightened expression in the intralaminar nuclei, whereas the $\alpha 3$ subunit displays the lowest expression level. Other subunits ($\beta 2$, $\beta 3$, $\beta 4$) are confined to specific thalamic nuclei. The $\alpha 7$ subunit is found throughout the thalamus in a pattern similar to that of $^3\text{H-}\alpha$ -bungarotoxin. Iontophoretic application or brain-stem stimulation-induced release of acetylcholine enhances spontaneous and stimulus-evoked discharges in relay neurons but suppresses them in GABAergic interneurons and reticular neurons. The excitation of relay neurons is mediated by both muscarinic and nicotinic receptors. The net effect of this increased activity is to block burst firing in hyperpolarized relay cells and bring them into a tonic firing mode. The acetylcholine-mediated inhibition of reticular neurons involves M2 muscarinic receptors. This inhibitory response in reticular neurons is preceded by a short nicotinic receptor-mediated depolarization similar to that found in relay cells. Stimulation of basal forebrain cholinergic nuclei inhibits reticular neurons.

The brain-stem pedunculopontine region is chemically heterogeneous and contains various populations of neurons that express acetylcholine, glutamate, or GABA. In monkeys, as many as 40% of cholinergic cells in this region coexpress glutamate. This high degree of cellular colocalization is reflected differently at the terminal level in various thalamic nuclei. For instance, many cholinergic terminals in centromedian (CM) and parafascicular (Pf) nuclei coexpress vGluTs, whereas less than 10% do so in the primate lateral geniculate nucleus. Similarly, almost 30% of cholinergic terminals in Pf express GABA, whereas less than 10% do so in the CM and lateral geniculate nucleus. These findings provide evidence for more complex and chemically diverse ascending brain-stem cholinergic inputs to the CM/Pf complex compared with relay thalamic nuclei in non-human primates.

7.5. Calcium-Binding Proteins

Thalamic neurons show differential expression of calcium-binding proteins, parvalbumin, calbindin D 28 k, and calretinin. In the primate thalamus, relay neurons stain for either parvalbumin or calbindin, but almost never for both. Calretinin is found in a subpopulation of calbindin neurons. Virtually no GABAergic interneurons express calcium-binding proteins, which is drastically different from other regions of the CNS, where inhibitory GABAergic interneurons are enriched in these proteins. In the primate thalamus, calbindin and parvalbumin show a marked reciprocity of distribution; for example, the thalamic nuclei strongly labeled for calbindin (anterior intralaminar nuclei, anterior pulvinar, laterodorsal nucleus, and medial geniculate nucleus) are usually devoid of parvalbumin immunoreactivity and vice versa. In the monkey dorsal thalamus, calbindin and parvalbumin together probably account for all relay cells. The caudal intralaminar nuclear group stands out among other thalamic nuclei for its complete lack of calbindin immunoreactivity. GABA cells in the reticular nucleus display strong parvalbumin immunoreactivity. Parvalbumin is detected in GABAergic terminals from the reticular nucleus in dorsal thalamic nuclei.

From the distributions of calbindin and parvalbumin in intralaminar and other nuclei outside of the principal sensory nuclei, Jones and colleagues have suggested that these nuclei contain a matrix of calbindin neurons that project diffusely to superficial layers of the cerebral cortex and, for some nuclei, a core of parvalbumin-enriched neurons whose cortical projections terminate preferentially in middle layers of cortex.

7.6. Neuropeptides

Many neuropeptides are found in afferent fibers to various thalamic nuclei. Among the most abundant neuropeptides identified thus far are tachykinin, cholecystokinin (CCK), somatostatin (SS), neuropeptide Y (NPY), neurotensin (NT), galanin, bombesin, angiotensin II (AII), enkephalin, and vasoactive intestinal peptide (VIP). Most thalamic peptides originate from brain stem and hypothalamic afferents. A few neuropeptides are expressed in thalamic neurons. Somatostatin is expressed in all thalamic reticular neurons. A small number of NPY-containing cells have been found in the ventral lateral geniculate nucleus. Angiotensin II appears to be widely expressed in the thalamus with highest concentrations in the sensory, mediodorsal, and anterior intralaminar nuclei. VIP has been found in some reticular neurons, and CCK-immunoreactive neurons have been identified in intralaminar and anterior nuclei. Apart from CCK, none of the thalamic peptides appear to be found in thalamocortical or corticothalamic projection systems. Although little is known about the function of these neuropeptides, the development of specific drugs combined with the cloning of various receptor subtypes provide useful tools to further understand the role of these neuromediators in the functional synaptic microcircuitry of the mammalian thalamus.

8. SPECIFIC CONNECTIONS OF THALAMIC NUCLEAR GROUPS

This section describes the organization of the main cortical and subcortical connections of thalamic nuclei. For simplification, thalamic nuclei are pooled into four major groups based on their cortical projections and localization: (1) the modality-specific nuclei, (2) the multimodal or association nuclei, (3) the non-specific or diffusely projecting nuclei, and (4) the epithalamus (Fig. 1).

8.1. The Modality-Specific Nuclei

This group includes six major thalamic nuclei: the anterior, ventral anterior, ventral lateral, ventroposterior, medial geniculate, and lateral geniculate nuclei.

8.1.1. THE ANTERIOR NUCLEI

In most animal species, the anterior nuclei comprise three distinct subdivisions: the anteromedial, anteroventral, and anterodorsal nuclei. This nuclear group is separated from the rest of the thalamus by

the internal medullary lamina. In humans, the principal anterior nucleus is the major component of this nuclear group. The laterodorsal nucleus shares many anatomic similarities with the anterior group. The anterior nuclei are part of the limbic system and play important roles in controlling emotional behavior, learning, and memory. All nuclei of this group have reciprocal connections with the cingulate cortex. They also project to suprasplenial and retrosplenial areas, extending as far as the parasubiculum. Afferents to the limbic thalamus arise from many sources, and the most important of these is the mammillary bodies that give rise to the mammillothalamic tract that ascends and terminates in various subdivisions of the anterior nuclei. Cells located in the medial part of the mammillary bodies project to the ipsilateral principal anterior nucleus, whereas fibers from the lateral mammillary body terminate in the anterodorsal nucleus bilaterally. Another main afferent to the limbic thalamus derives from the subiculum and presubiculum region of the hippocampal formation. The basolateral, basomedial, and lateral amygdala nuclei are other sources of inputs to the anteromedial nucleus.

Acetylcholinesterase labeling is very dense in the anterodorsal and anteroventral nuclei, slightly lower in the laterodorsal nucleus, and nearly absent from the anteromedial nucleus. The anteroventral nucleus expresses the highest thalamic levels of muscarinic cholinergic receptors and receives the densest brainstem cholinergic innervation, mostly from the laterodorsal tegmental nucleus.

Despite their close interconnections with so-called limbic-related structures, lesions of anterior nuclei or limbic cortex have greater impact on spatial learning and memory than on emotional responses, similar to those associated with hippocampal lesions. Anterior nuclei neurons, most particularly those in the anterodorsal nucleus, also seem to play a role in regulating “head direction,” at least in rodents. The laterodorsal nucleus is involved in spatial processing. Consistent with their role in memory functions, most

thalamic plaques and tangles in Alzheimer’s disease are found in anterior nuclei. Degeneration of the anterior nuclei is reported in Korsakoff’s psychosis, a disease characterized by a loss of anterograde memory (see later).

8.1.2. THE VENTRAL ANTERIOR AND VENTRAL LATERAL NUCLEI

The ventral anterior and ventral lateral nuclei are the main targets of basal ganglia and cerebellar inputs to the thalamus. These two nuclear groups are divided into various subnuclei that are based on cyto-logic criteria. The nomenclature of these nuclei is extremely confusing, which makes understanding of papers published in this field difficult for nonspecialists (Table 1 and Table 2). Here, we use the nomenclature of Jones (1985, 2007) to describe projections of these nuclei. According to this nomenclature, the ventral anterior (VA) nucleus is the main recipient of GABAergic inputs from the substantia nigra pars reticulata (SNr), the anterior ventrolateral nucleus (VLa) receives inputs from the internal globus pallidus (GPi), whereas the posterior ventrolateral nucleus (VLp) is the main target of ascending cerebellar afferents. There is little or no overlap between these three projection systems in the primate thalamus. On the other hand, a slightly higher degree of convergence of cerebellar and basal ganglia inputs is found in rats and cats, though this remains controversial (Jones, 2007). Both SNr and GPi projections are ipsilateral, use GABA as neurotransmitter, and terminate on the proximal part of thalamocortical neurons and presynaptic dendrites, whereas cerebellar inputs are glutamatergic and arise from the contralateral dentate nucleus. Because of these close relationships with motor-related subcortical regions, the VA/VL nuclei are often termed *motor thalamic nuclei*.

In contrast with the long-held belief that basal ganglia outflow was conveyed exclusively to premotor (PM) and supplementary motor (SMA) cortical areas, it is now established that a substantial contingent of information from the basal ganglia is sent to

Table 1
Nomenclature of Various Subdivisions of the VA/VL Nuclear Complex in New World and Old World Monkeys*

Olszewski	Vlo	VPLo	Area X	Vlc (and VLps)	VLM	VApC	Vamc
Jones	VLa			VLp	VMp		VA
Illinsky and Kultas-Illinsky	VAdc	VL	VLD	VM	VApC	Vamc	
Paxinos et al.	VAL (Vo)	VLL	VLM	VAL	VAM	VAL (Vo)	
Stepniewska et al.	VLa	VLp	VLx	VLD	VM	VApC	Vamc

Table 2
Correlation Between Nomenclature of Ventral Thalamic Nuclei in Humans and Two Commonly Used Nomenclatures in Macaque Monkey

<i>Human/monkey</i>	<i>Jones</i>	<i>Olszewski</i>
N. ventro-caudalis anterior internus (V.c.a.i.)	VPM	VPM
N. ventro-caudalis portae (V.c.por)	Pla	Plo
N. ventro-caudalis parvocellularis internus (V.c.pc.i.)	VMb + Sm	VPMpc
N. ventro-caudalis parvocellularis externus (V.c.pc.e)	VPI	VPI
N. zentrolateralis caudalis (Z.c.)	VPLa (posteriorodorsal)	VLL (part)
N. Ventr-intermedius (V.im)	VLp (ventral part)	VPLo
N. dorso-intermedius (D.im)	VLp (dorsal part)	VLC
N. zentro-intermedius (Z.im)	VLp/VPLa (parts)	VLC/VPLc (parts)
N. ventro-oralis anterior (V.o.a)	VLa	—
N. ventro-oralis posterior (V.o.p)	VLa/part VLp	Vlo
N. ventro-oralis medialis (V.o.m)	VM	VLM
N. ventro-oralis internus (V.o.i)	VLp (anteromedial)	Area X
N. zentrolateralis oralis (Z.o)	VLa (parts)	Vlo (parts)
N. dorso-oralis (D.o)	VA (parts)	—
N. lateropolaris (L.po)	VA	VA
N. lateropolaris magnocellularis (L.po.mc)	VAmc	VAmc

the primary motor cortex (M1). Conversely, the cerebellar outflow, which was believed to be directed exclusively to M1, also reaches PM and SMA cortical regions. Cells that project to M1 are found in all parts of VLp, whereas those that innervate the SMA are most particularly confined to the so-called area X, a subregion of cerebellar VLp defined by Olszewski, and VLa. All VLp cells that project to M1 are glutamatergic and express parvalbumin immunoreactivity.

All three deep cerebellar nuclei project to VLp. The dentate and interposed nuclei project mainly on the contralateral side, whereas the fastigial nucleus provides bilateral projections to the thalamus. The dentate gives rise to the heaviest projection that innervates homogeneously all parts of VLp, whereas projections from the interposed nucleus are more dispersed and those from the fastigial are the least prominent and widely dispersed throughout the nucleus. These projections are highly topographic, the anterior part of all cerebellar nuclei being connected mainly with the lateral VLp, whereas the posterior part of these nuclei project to the medial VLp. On the other hand, the lateral division of the deep cerebellar nuclei project dorsally in VLp, whereas the medial division innervates the ventral tier of VLp. Both anatomic and single-unit studies confirmed that projections from the dorsal part of the dentate nucleus display a lamellar somatotopic organization in the monkey VLp (Fig. 4).

Pallidal projections to VLa are functionally topographic, use GABA as transmitter, and terminate in a patch-like pattern, like most VL inputs. The functional organization of the pallidothalamic projection is highly dependent on the source of striatal neurons that impinge upon pallidothalamic neurons that contribute to this projection. For instance, neurons in the ventro-posterior two-thirds of GPi receive sensorimotor striatal innervation, dorsal third GPi neurons receive associative inputs from the caudate nucleus, and anteromedial GPi neurons receive predominately limbic inputs from the ventral striatum. This pattern of organization led Alexander and colleagues (1986) to propose the concept of functionally segregated cortico-striato-pallido-thalamo-cortical loops throughout the basal ganglia circuitry. This concept has been revised, fine tuned, and challenged over the past 20 years, but it appears that overall, the information flow through the basal ganglia–thalamocortical circuitry from functionally segregated cortical regions is mainly processed along separate channels. Cross-talks between these different channels are likely mediated by cortico-cortical connections. It is worth noting that the functional specificity of these connections is dramatically reduced in dopamine-depleted brains of parkinsonians. The VLa is not microexcitable, which makes it different from VLp. VLa neurons are silent at rest, probably due to tonic inhibition from basal ganglia output nuclei. Although some GPi-receiving neurons in VLa

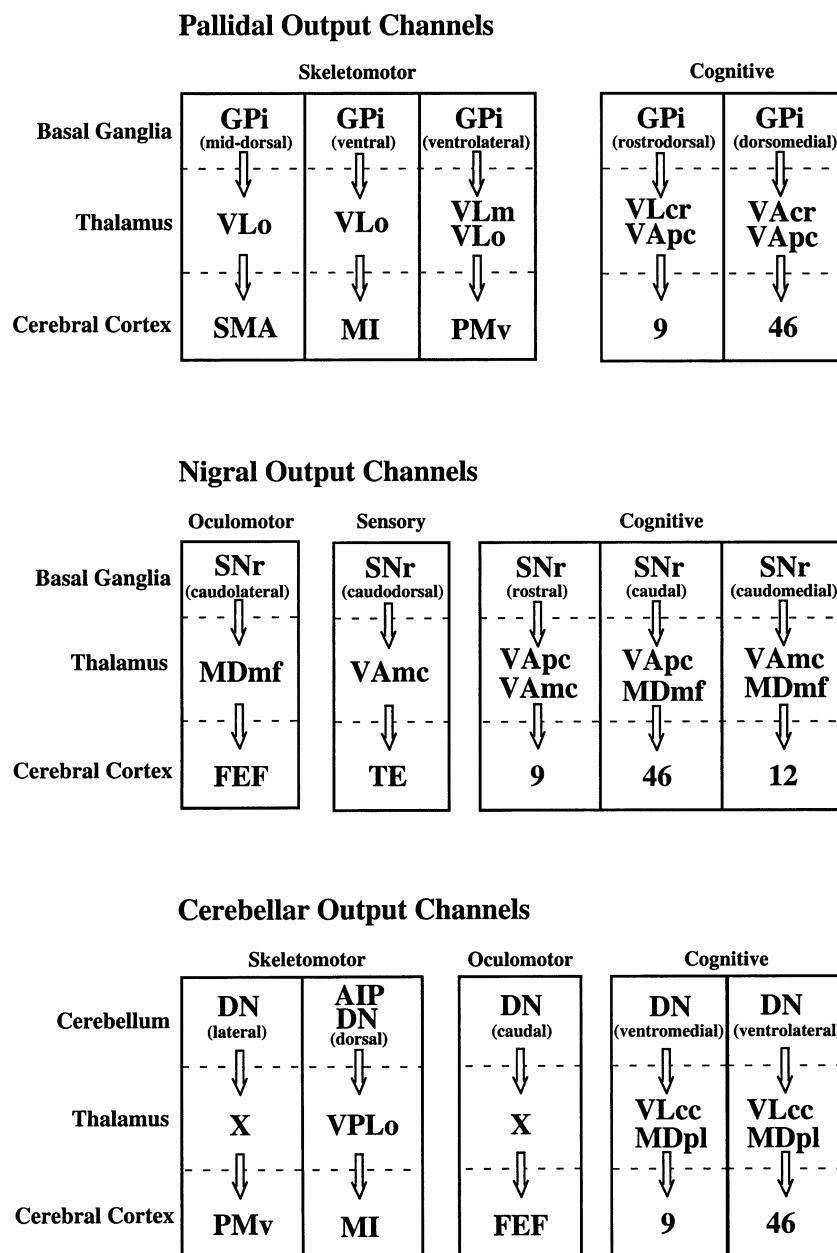


Fig. 4. Segregated basal ganglia and cerebellar motor and nonmotor thalamocortical output channels in monkeys. The GPi, SNr, and deep cerebellar nuclei project to different subdivisions of VA/VL and MD nuclei, which, in turn, reach functionally segregated cortical areas involved in motor, cognitive, and sensory functions. The nomenclature of thalamic nuclei used in this diagram is that of Olszewski (*see* Table 1 for abbreviations). AIP, interpositus nucleus; FEF, frontal eye field; MDmf, mediodorsal nucleus, pars multiformis; MDpl, mediodorsal nucleus, pars lateralis; TE, area of inferotemporal cortex. (Reproduced from Middleton FA, Strick PL. Basal ganglia and cerebellar loops—motor and cognitive circuits. *Brain Res Rev* 2000;31:236–250, with permission from Elsevier Science.)

change their firing rates in response to movements, they are not as tightly synchronized with movements as are cerebellum-receiving VLp neurons (Fig. 4).

Both cerebellar and basal ganglia thalamic projections terminate in motor thalamic territories but also reach major associative and limbic regions of the

primary thalamus, which, in turn, innervate various associative cortical areas in the frontal, parietal, and temporal lobes involved in cognitive functions. These projections provide a substrate by which basal ganglia and cerebellar functions extend beyond motor control and involve complex cognitive and learning processes.

The ventral anterior and mediodorsal thalamic nuclei are the main targets of nigrothalamic projections. In monkeys, inputs from the medial part of the SNr terminate mostly in the medial magnocellular division of the VA (VAmc) and the mediodorsal nucleus (MDmc), which, in turn, innervate anterior regions of the frontal lobe, including the principal sulcus (Walker's area 46) and the orbital cortex (Walker's area 11). Neurons in the lateral SNr project preferentially to the lateral posterior region of the VAmc and to different parts of MD that are mostly related to posterior regions of the frontal lobe, including the frontal eye field and areas of the premotor cortex. Nigral outputs to the thalamus flow along separate channels that target various cortical areas involved in cognitive, sensory, and oculomotor functions. SNr projections also target the ventromedial nucleus (VM) (Fig. 4).

The spinothalamic tract also extends some projection fibers to the VLp, being mainly concentrated in the ventral tier of the nucleus in monkeys. These fibers arise from both superficial and deep dorsal horn spinal cord neurons. Consistent with such a pathway, neurons with passive cutaneous receptive fields have been identified in the ventral, but not the dorsal sectors of the VLp, which may provide the basis for short-latency somatosensory information to reach motor cortex. Tectal and vestibular inputs to VLp from the pretectum region, superior colliculi, or vestibular nuclei have also been identified.

8.1.3. THE VENTROPOSTERIOR AND VENTROMEDIAL NUCLEI

The ventroposterior nucleus is the main recipient of ascending somatosensory information. Two major subnuclei are recognized based on topographic and cytoarchitectonic organization: the ventroposterolateral (VPI) and ventroposteromedial (VPm) nuclei. In both nuclei, cells are arranged in clusters involved with the same modality and receptive fields. The main sources of inputs to the VPI are the medial lemniscus and spinal lemniscus, which carry sensory information related to the extremities and the trunk. Terminations of the medial lemniscus and spinothalamic tracts end on different targets in VPI. Medial lemniscal afferents contact projection neurons and interneurons and represent major components of complex synaptic arrangement, whereas spinothalamic fibers almost exclusively innervate projection neurons and avoid complex synaptic arrangements. The main input to the VPm arises from the trigeminal lemniscus that carries all somatic sensory modalities

of the face. The gustatory sense is also represented in the VPm. Both VPm and VPI contain precise somatotopic maps of corresponding body parts. Lemniscal axons terminate in a rod-like fashion that encompasses clusters of thalamic neurons. Terminals within each rod carry a specific somatosensory modality that is transferred to specific columns of the primary somatosensory cortex. These precise connections provide the basis for preserving the somatotopic organization within the somatosensory system. The main cortical projection sites of VPI and VPm is the primary somatosensory cortex that comprises Brodmann's areas 1, 2, 3a, and 3b in the postcentral gyrus. In turn, these cortical regions provide inputs to the ventroposterior nucleus. Additional inputs from VPI and VPm to nonsomatosensory cortical regions of the parietal lobe have also been shown.

The ventral medial (VM) nucleus is an integral part of the ventroposterior nuclear group that appears as an extension of VPm. The main ascending inputs to this nucleus come from the gustatory division of the parabrachial nucleus of the pons and possibly from the nucleus of the solitary tract. In monkeys, the nucleus is divided into an anterior nongustatory division and a posterior gustatory part. The VM projects to an area of cortex located between SI and the insula. Stimulation of the VM in humans induces sensation of taste and gastric fullness plus painful and nonpainful somatic sensations. Based on connectivity and functional studies, three visceral pathways run through the ventroposterior complex: (1) a special visceral pathway for taste through the medial VM, (2) a general visceral pathway through the lateral VM, and (3) a further visceral pathway, perhaps predominately vagal, through the medial VM (see Jones [2007] for review).

8.1.4. THE MEDIAL GENICULATE NUCLEUS

The medial geniculate nucleus is cytoarchitectonically subdivided into three main parts in primates. These regions are termed *ventral*, *dorsal*, and *magnocellular*. The ventral nucleus is the primary auditory relay to the primary auditory cortex located in Brodmann's areas 41 and 42 in Heschl's gyri. There is a point-to-point representation, called a tonotopic map, of the cochlea in this region. Neurons show high-frequency tuning and respond preferentially to inputs from the contralateral ear, although inputs from the ipsilateral ear also influence these neurons. Inputs to this subnucleus arise from the contralateral cochlea, with only one synaptic relay in the central nucleus of the inferior colliculus, which then sends fibers through the brachium of the

inferior colliculus. The dorsal and magnocellular nuclei receive less direct auditory inputs than does the ventral nucleus; neuronal responses in these regions are less frequency-specific, and the tonotopic maps are not well-defined, or even absent. The dorsal subnucleus receives auditory inputs from various brain-stem nuclei and projects outside, but around, the primary auditory cortex. On the other hand, the magnocellular region receives auditory, somatosensory, and vestibular afferents by collaterals of the spinothalamic, medial lemniscal, and brachium of the superior colliculus tracts and provides more widespread projections to nonauditory cortical areas than do the two other subnuclei.

8.1.5. THE LATERAL GENICULATE NUCLEUS

The lateral geniculate nucleus (LGN) is the main thalamic center for processing visual information. It is one of the most distinctive nuclei of the thalamus by its lamination into six distinct layers that receive completely segregated inputs from the two eyes. The ventral-most two layers (1 and 2) are composed of large cells and are called *magnocellular layers*, whereas the dorsal layers (3 to 6) comprise small cells, thereby called *parvicellular layers*. The major afferent inputs to the lateral geniculate nucleus arise from the retina. The axons of retinal ganglion cells travel through the optic tract and terminate in an orderly fashion into specific layers of the LGN, preserving a point-to-point map of the visual space. Layers 2, 3, and 5 receive inputs from the ipsilateral eye, whereas layers 1, 4, and 6 are the main targets of the contralateral eye. The information is then conveyed to primary and secondary visual areas (Brodmann's areas 17 to 19) in the occipital lobe. The terminations of fibers that arise from the LGN laminae and are innervated by the ipsilateral and contralateral eyes do not overlap in the primary visual cortex. Instead, they terminate in a series of alternate left- and right-eye domains of 400 to 500 μm wide—known as ocular dominance columns—primarily in cortical layer IV. A main feature of LGN neurons is their center-surround receptive field, first described in the primary visual cortex by Hubel and Wiesel in the 1960s. In brief, a center-surround receptive field means that cells have a central zone in which a flash of light elicits excitation (on-center) or inhibition (off-center) of the cell, and a concentric surround in which flashed-light stimuli inhibit an on-center cell or excite an off-center cell. The LGN GABAergic interneurons play a major role in defining the extent of these receptor fields. Most retinal ganglion cells

can be divided into two main categories based on the functional organization of their center-surround receptive fields. The M or P- α cells (parasol ganglion cells), which account for about 10% of the total ganglion-cell population, are not color-coded, display a broad-band spectral sensitivity, and have large concentric receptive fields. They respond to changes in brightness and have fast-conducting axons. The P or P- β cells (midget cells) have much smaller receptive fields, are color-coded, and are thereby excited by certain wavelengths and inhibited by others. Overall, the axons of M and P cells are segregated among laminae of the LGN; the M cells exclusively innervate the magnocellular layers 1 and 2, whereas the P cells terminate preferentially in the parvocellular layers. Another way to categorize retinal ganglion cells is by their pattern of innervation and neuronal targets in the LGN. The so-called X-type retinal axons are involved in complex synaptic associations, known as glomeruli, with relay neurons and interneurons, whereas the Y-type axons terminate directly on relay neurons and are usually not part of synaptic glomeruli. Additional afferents to the LGN come from primary and secondary visual cortical areas, the superior colliculus, the pretectal nucleus, brain-stem oculomotor regions, and tegmental monoaminergic and cholinergic cell groups.

8.2. The Multimodal Association Nuclei

As mentioned previously, these nuclei differentiate themselves from the modality-specific nuclei by their diversity in sources of innervation and widespread cortical projections to association cortices. The major association nuclei of the primary thalamus are the mediodorsal and lateral posterior-pulvinar nuclei.

8.2.1. THE MEDIODORSAL NUCLEUS

The mediodorsal nucleus is a large nuclear mass located dorsomedial to the internal medullary lamina. It is tightly connected with the prefrontal agranular cortex and plays major roles in high-order cognitive functions. In primates, the MD is cytoarchitectonically divided into three subnuclei: a medial, magnocellular nucleus; a parvocellular nucleus, which encircles the magnocellular compartment; and a lateral, multiform nucleus. Olfactory inputs from the prepiriform cortex and olfactory tubercle as well as the amygdala are considered to be the main sources of afferents to the magnocellular region of the nucleus. GABAergic inputs from basal ganglia

nuclei—such as the ventral pallidum and SNr—terminate in all subdivisions of the nucleus, whereas the parvocellular and multiform subnuclei receive additional inputs from the superior colliculus, midbrain tegmentum, and brain-stem monoaminergic and cholinergic neurons. Each subnucleus has a preferential termination site in the prefrontal cortex: the magnocellular nucleus projects mainly to orbital areas (Brodmann's areas 10, 11, 12, 13), and projections from the parvocellular nucleus are more widespread and include lateral and medial areas of the frontal lobes (Brodmann's areas 9, 24, 32, 45, 46, etc.). Finally, the multiform nucleus innervates preferentially more caudal regions of the frontal lobe, particularly the frontal-eye field. In line with these anatomic connections, neurons in the magnocellular MD and the orbital prefrontal cortex are activated after electrical stimulation of the olfactory cortex or olfactory bulb and respond to odors. The MD's tight connections with the prefrontal cortex explain why it is involved in various cognitive brain diseases such as schizophrenia and Korsakoff's syndrome, which are characterized by changes in emotional behavior and loss of memory.

8.2.2. THE LATERAL POSTERIOR/PULVINAR NUCLEI

The lateral posterior/pulvinar complex is much larger and more highly differentiated in primates than in any other animal species. In monkeys and humans, one lateral posterior nucleus and four pulvinar subdivisions (anterior, lateral, medial, and inferior) have been identified. The main afferent inputs to this nuclear group arise from visuomotor regions of the midbrain, especially the superficial and deep layers of the superior colliculus and pretectum. There is also a direct retinal input to the inferior pulvinar nucleus. The superficial layers of the superior colliculus provide an indirect route through which retinal inputs reach these nuclei. Deep layers of the superior colliculus are likely to provide motor-related inputs from the basal ganglia, cerebellum, and brain-stem centers that process various sensory modalities. Cortical projections arise from widespread regions of the parieto-temporo-occipital cortices. The inferior and lateral pulvinar nuclei contain one or more representations of the contralateral visual fields, probably as a result of their topographically organized projection from the superior colliculus. Based on these connections, the lateral posterior nucleus-pulvinar complex is often considered to be an integrative center for sensory and motor information related primarily to vision.

8.3. The Nonspecific or Diffusely Projecting Nuclei

This nuclear group includes two major sets of thalamic nuclei named for their location in the thalamus. The intralaminar nuclei are found within the internal medullary lamina, whereas the midline nuclei lie along the medial wall of the thalamus, just along the third ventricle. The main feature of these nuclei is that they provide rather diffuse projections to the cerebral cortex and project mainly to the striatum and other subcortical regions.

8.3.1. THE INTRALAMINAR NUCLEI

The intralaminar nuclei comprise two major groups, namely the anterior and posterior intralaminar nuclei. Two main nuclei are recognized in the anterior group, the paracentral and the centrolateral, whereas the posterior group comprises the centromedian (CM) and parafascicular (Pf) nuclei. The main projection site of the caudal intralaminar nuclei is the striatum, whereas rostral nuclei innervate preferentially the cerebral cortex with significant axon collateralization to the striatum. The organization and function of the thalamostriatal system and its place in the functional circuitry of the basal ganglia are discussed in a separate section.

Other subcortical targets of intralaminar nuclei include the amygdala, substantia innominata, globus pallidus, subthalamic nucleus, and claustrum. Although less massive than thalamic inputs to the striatum, intralaminar nuclei also project to various cortical regions. Cortical projections from the anterior intralaminar nuclei are partly collaterals of the thalamostriatal pathways, whereas cortical and striatal afferents from CM/Pf largely arise from segregated neuronal populations. Although considered to be diffuse and primarily confined to layer I of the cortex, it appears that clusters of cells in intralaminar nuclei project to relatively restricted cortical areas and that layer VI also receives some intralaminar projections. Based on data largely obtained in non-primates, the cortical projections of intralaminar nuclei are organized as follows: anterior intralaminar nuclei project mainly to various functional areas in the prefrontal, cingulate, parietal, temporal, prepiriform, and entorhinal cortices as well as the hippocampus; the CM is reciprocally connected with motor and somatosensory cortical regions, and the Pf projects to prefrontal, premotor, and cingulate cortices.

The anterior intralaminar nuclei receive subcortical inputs from various brain stem, cerebellar, and spinal cord nuclei. Additional inputs from the

amygdala substantia nigra, superior colliculus, and pretectal nuclei have also been found. The CM/Pf are the main targets of basal ganglia projections from GPi and SNr, which largely arise from collaterals of the basal ganglia outputs to the VA/VL. Brain stem cholinergic and monoaminergic inputs from the pedunculopontine nucleus, raphe nuclei, and locus coeruleus have also been established. Notably, projections from the pedunculopontine nucleus are mainly directed toward Pf and display a high degree of chemical heterogeneity using acetylcholine, GABA, and glutamate as coexisting neurotransmitters. The reticular formation (RF) also provides massive inputs to anterior and posterior intralaminar nuclei. By virtue of these strong associations with the RF, the intralaminar nuclei are traditionally seen as part of the *reticular activating system* that regulates the mechanisms of cortical arousal and attention. Other functions of intralaminar nuclei include the regulation of tolerance to pain and motor control mediated by spinothalamic and basal ganglia afferents, respectively.

8.3.2. THE MIDLINE NUCLEI

The midline nuclei comprise three main cell groups that are much better defined in non-primates than in monkeys and humans. The most prominent nuclei are the paraventricular, rhomboid, and reunions nuclei. The bulk of efferents from these regions are directed toward limbic-related cortical and subcortical areas. Similarly, they receive inputs from various limbic structures such as the amygdala and hypothalamus, as well as several midbrain and medullary regions including the nucleus of the solitary tract (NST), the periaqueductal gray, the parabrachial region, the raphe nucleus, and the locus coeruleus. Collaterals of the spinothalamic tract also reach these nuclei. By virtue of their pattern of connectivity, midline thalamic nuclei are likely to be involved in emotional and motivational behaviors and autonomic functions.

8.4. The Epithalamus

8.4.1. THE PARAVENTRICULAR NUCLEUS

The epithalamus comprises the anterior and posterior paraventricular nuclei, the medial and lateral habenular nuclei, the stria medullaris, and the pineal gland. The pineal gland will not be discussed further in this chapter. These regions have a common origin from the dorsal diencephalic neuroepithelium during thalamic development.

The paraventricular nucleus (PVN) is made up of small, densely packed cells divided into the anterior and posterior nuclei that lie just beneath the ventricular system ependyma from which they are separated by thin fibers named stratum zonale. Caudally, the posterior PVN turns ventrally to the habenular nuclei and down along the posterior pole of the mediodorsal nucleus to enter the mesencephalic periaqueductal gray matter of the midbrain with which it merges. The PVN displays a pattern of transmitters and neuropeptides expression different from that of other dorsal thalamic nuclei, but very similar to that of the habenular nuclei, which supports the view that these two nuclei have a common origin. In brief, some of the most abundant chemicals in PVN are acetylcholinesterase and limbic-associated glycoprotein (LAMP), enriched in neuropil and some cell bodies, dopaminergic, noradrenergic, and serotonergic fibers from brainstem monoaminergic nuclei, including the ventral tegmental area (VTA) as the main source of dopaminergic inputs, cholinergic fibers from PPN, and brainstem enkephalin terminals. Other neuropeptides identified in these nuclei include neuropeptide Y, somatostatin, cholecystokinin, and vasoactive intestinal peptide. All PVN neurons express calretinin, some of which, in the middle and ventral tiers of the nucleus, also display calbindin D28k immunoreactivity. There are no parvalbumin cells and fibers in the PVN. The PVN is also devoid of GABAergic interneurons but receives GABAergic innervation. In contrast with all dorsal thalamic nuclei, the PVN does not express CAMKII- α . The pattern of glutamate and GABA receptors expression is very similar to that of the habenular nuclei but, for specific subunits, different from that of other thalamic nuclei.

Inputs to the PVN originate from the suprachiasmatic nucleus of the hypothalamus, limbic cortex, ventral lateral geniculate nucleus, zona incerta, ventral pallidum, amygdala, bed nucleus of stria terminalis, and brainstem monoaminergic and cholinergic cell groups. PVN efferents terminate predominately in various limbic cortices and ventral striatum (shell of the nucleus accumbens). Other targets of PVN neurons include the suprachiasmatic nucleus, dorsomedial and ventromedial hypothalamic nuclei, bed nucleus of stria terminalis, amygdala, olfactory tubercle, and endopiriform nucleus.

Although not much is known about the exact functions of PVN, various experimental data suggest its role in (1) autonomic functions, (2) behavioral responses to psychoaffective drugs, (3) circadian rhythm, and (4) processing of pain and stressful stimuli.

8.4.2. THE HABENULAR NUCLEI

The lateral and medial habenular nuclei are located in the posterodorsal part of the thalamus, just beneath the ependyma of the third ventricle. The two nuclei, originally named by Nissl, are found in all vertebrates but have undergone significant cytologic differentiation across species. For instance, the medial habenular nucleus is larger than the lateral nucleus in monotremes, but such is not the case in humans. The medial nucleus is made up of numerous small, darkly stained neurons; whereas the lateral nucleus, commonly larger than the medial nucleus, consists of paler, more diffusely distributed cells. Cytologically, the lateral nucleus comprises two parts: the lateral magnocellular division and the medial parvocellular region. Two populations of cells have been identified in the lateral habenula; a group of large neurons that send axons into the habenulointerpeduncular tract and a population of small cells that likely represent interneurons. On the other hand, the medial habenula is made up of a single population of bushy cells that project their axons into the habenulointerpeduncular tract.

The three main calcium-binding proteins display unique and complementary patterns of distribution in the medial and lateral habenular nuclei. Whereas parvalbumin is expressed in both nuclei, calbindin and calretinin are much more abundant in the medial habenula. Very few GABA-containing cells are found in either habenular nuclei, but both GABA_A and GABA_B receptors are strongly expressed. Cholinergic neurons, and to a lesser extent substance P-immunoreactive cells, which make up the medial habenular nucleus, are the main source of axons in the habenulointerpeduncular projection.

Afferents to the habenular nuclei mainly arise from the stria medullaris. The main sources of inputs to the lateral habenula include the prepiriform cortex, GPi, and the basal nucleus of Meynert. Other, more modest, inputs arise from the preoptic area, the lateral hypothalamus, the VTA, the laterodorsal tegmental nucleus, the SNC, the periaqueductal gray, and the raphe nucleus. The medial habenula receives most of its innervation from calretinin- and calbindin-containing neurons in the posterior septum. In primates, pallidohabenular projections terminate in the magnocellular lateral habenular nucleus, whereas other inputs innervate predominately the parvocellular region of the lateral habenula. Other chemically characterized inputs to the habenula include brainstem monoaminergic afferents from the raphe and the locus coeruleus, enkephalin-immunoreactive fibers as

well as Luteinizing Hormone-Releasing Hormone (LHRH) and Brain-Derived Neurotrophic Factor (BDNF) containing fibers from the bed nucleus of the stria terminalis.

Various functions have been associated with the habenula mainly through behavioral observations in lesioned animals. A series of endocrine and visceral functions have been recognized. Altogether, data obtained to date suggest that the habenular nuclei are involved in regulation of sensitization to drugs, nociception, sleep, hoarding behavior, hormonally dependent reproductive behavior, and motor exploration. In particular, the lateral habenular nucleus is implicated in stress responses, maternal behavior, reward behavior, and reproductive behavior. Lateral habenular neurons also play a critical role in regulating activity of midbrain dopaminergic neurons of SNC and VTA. Electrical stimulation of the lateral habenula profoundly inhibits SNC dopaminergic neurons, whereas habenular lesions increase the rate of spontaneous firing of midbrain dopaminergic neurons. This projection plays an important role in mediating inhibitory responses of SNC dopaminergic neurons to peripheral nociceptive stimulation. Furthermore, recent *in vivo* electrophysiology data demonstrated an important role of this system in regulating reward-related responses of SNC neurons in non-human primates.

9. THE CORTICOTHALAMIC SYSTEM

The dorsal thalamic nuclei and the cerebral cortex are reciprocally linked through thalamo-corticothalamic loops, characterized by the tight topography between thalamocortical inputs to a specific and restricted cortical sector, which, in turn, provides a significant feedback projection to the same thalamic region that contributed the cortical innervation. However, this accounts for only part of the corticothalamic system, which provides a much more massive and widespread innervation of the thalamus than previously thought. The corticothalamic fibers spread beyond the confines of the zone of thalamic relay neurons from which originate the reciprocal thalamocortical projections. This nonreciprocal corticothalamic system originates from layer V pyramidal cortical neurons, whereas layer VI neurons are the main sources of reciprocal inputs to the thalamus. Although the functional significance of the nonreciprocal corticothalamic system remains to be elucidated, it is reasonable to suggest that these connections mediate communication between thalamo-cortico-thalamic circuits (Jones, 2007).

10. THE THALAMOSTRIATAL SYSTEMS

10.1. Anatomic and Synaptic Organization of the Thalamostriatal Systems

The caudal intralaminar nuclei, centromedian (CM) and parafascicular nuclei (Pf), are the main sources of the thalamostriatal projection. The CM projects preferentially to the postcommissural putamen (the sensorimotor striatal territory), whereas the Pf innervates preferentially the precommissural putamen, the caudate nucleus, and nucleus accumbens (the associative and limbic striatal territories, respectively). The anterior intralaminar nuclei project mainly to the nucleus accumbens. Other relay and associative thalamic nuclei also contribute significantly to the thalamostriatal projections in primates and non-primates (Fig. 5).

The thalamostriatal projection system is glutamatergic, enriched in vGluT2, and terminates preferentially in the striatal matrix compartment, except for inputs from the paraventricular and rhomboid nuclei that preferentially terminate into the patch compartment of the ventral striatum. It appears that thalamic inputs from CM preferentially target a subpopulation of striatal projection neurons that innervate the

internal globus pallidus (GPi), so-called direct striatofugal neurons. This represents an evidence that an extrinsic input to the striatum targets a subpopulation of striatofugal neurons. In turn, the CM and Pf are the main targets of basal ganglia outputs from GPi and SNr. Recent anatomic studies show that these projections are massive and display a high level of functional specificity. For instance, the sensorimotor, associative, and limbic territories of GPi innervate segregated regions of CM/Pf; the sensorimotor inputs are confined to the CM, and the associative and limbic GPi project mainly to Pf. This specific pattern of functional organization of basal ganglia–thalamostriatal connections indicates that information flows through these circuits in segregated parallel channels, a common feature for processing of information in the basal ganglia (Fig. 6).

At the ultrastructural level, there are striking differences in the pattern of synaptic innervation of striatal projection neurons by inputs from CM/Pf compared with other thalamic nuclei. While thalamic boutons from CM/Pf form asymmetric synapses predominately with dendrites of projection neurons and interneurons, thalamic inputs from rostral intralaminar, midline, mediiodorsal, laterodorsal,

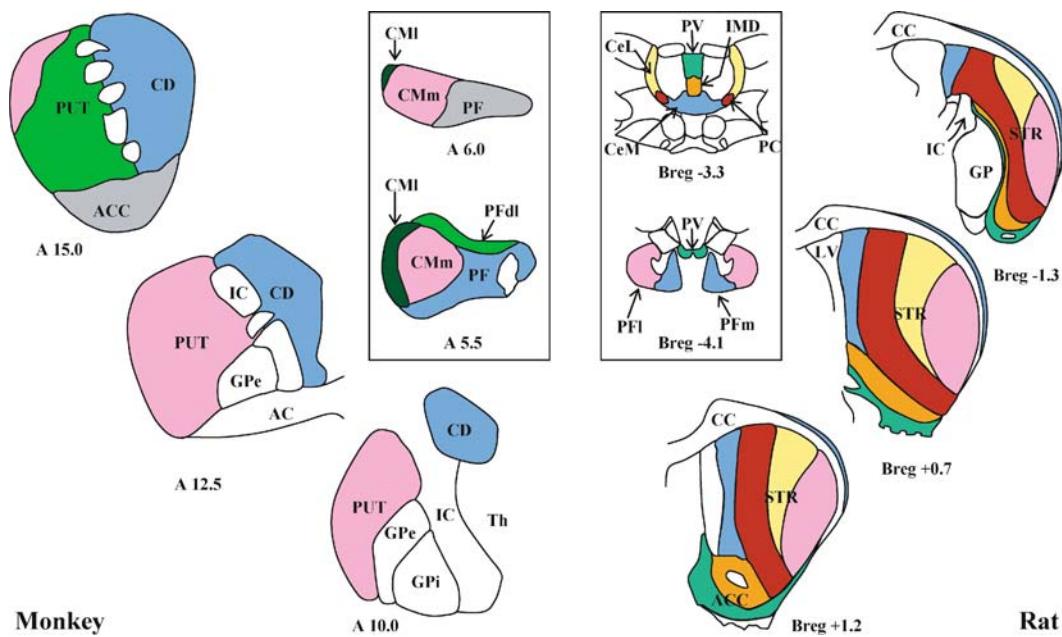


Fig. 5. Pattern of distribution of thalamic inputs to the rostrocaudal extent of the (A) monkey and (B) rat striatum. Striatal territories receive afferents from gray tone-coded thalamic nuclei. The lateral part of CM (CMI) in monkey projects preferentially to the primary motor cortex. AC, anterior commissure; Acc, nucleus accumbens; CC, corpus callosum; Cd, caudate nucleus; CeL, centrolateral nucleus; CeM, centromedial nucleus; CMm, medial CM; GP, globus pallidus; GPe, globus pallidus, external segment; GPI, globus pallidus, internal segment; IC, internal capsule; Imd, intermediodorsal nucleus; LV, lateral ventricles; PC, paracentral nucleus; PF, parafascicular nucleus; PFdl, dorsolateral PF; PFI, lateral PF; PFm, medial PF; Put, putamen; PV, paraventricular nucleus; Str, striatum; Th, thalamus.

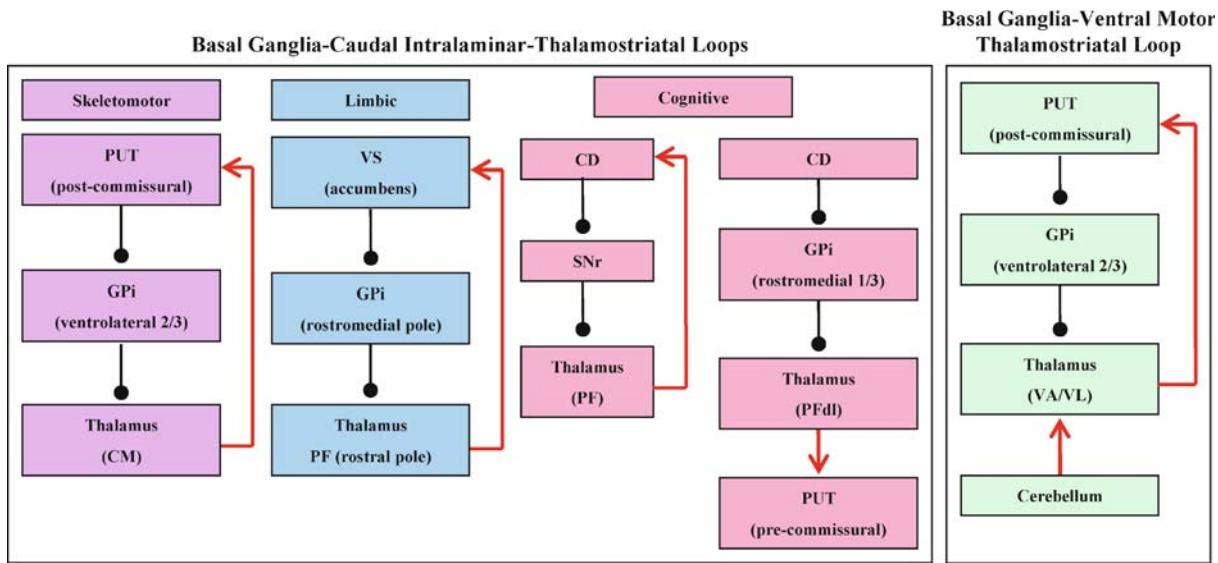


Fig. 6. Basal ganglia–thalamostriatal loops through the caudal intralaminar and ventral motor thalamic nuclei in monkeys. DOT connections, inhibitory GABAergic; ARROW connections, excitatory, glutamatergic (see Fig. 5 for abbreviations).

anteroventral, and VA/VL almost exclusively contact dendritic spines in the rodent striatum. Another main difference between thalamic inputs from CM/Pf versus those from other thalamic nuclei is their degree of synaptic interactions with dopaminergic afferents. Whereas axo-spinous thalamic afferents and dopamine terminals often converge on the same postsynaptic spines, thalamic boutons from CM and dopamine terminals are never found in close proximity to each other in the monkey striatum. This suggests that the dopaminergic inputs are located to subserve a more specific control of axo-spinous thalamic afferents than do axo-dendritic thalamic influences from CM/Pf.

10.2. Thalamostriatal Versus Thalamocortical Systems: Do They Have a Segregated or Common Origin?

Overall, there is agreement among retrograde double-labeling studies that a substantial proportion of neurons in the rostral intralaminar nuclear group and some specific thalamic nuclei (VA/VL, mediodorsal nucleus) provide axon collaterals to both the striatum and the cerebral cortex, whereas thalamostriatal and thalamocortical neurons are largely segregated in the caudal intralaminar CM/Pf nuclear complex. For instance, CM projections to the primary motor cortex in monkeys arise preferentially from a restricted neuronal population confined to lateral part of CM, whereas neurons in medial CM project to the postcommissural putamen. However, single-cell filling injection data suggest that most, if

not all, Pf neurons that project to the caudate-putamen complex send sparse collaterals to the cerebral cortex in rats. This pattern is opposite that displayed by projections from the centrolateral nucleus, which provide scarce, loosely organized, long varicose processes to the striatum but form dense patches of terminals in the rat cortex. Therefore, it appears that the thalamostriatal system from the CM/Pf is different than other thalamostriatal projections in the degree of divergence to the cerebral cortex. For the most part, inputs from CM/Pf terminate preferentially and more densely in the striatum with scarce diffuse collaterals to the cerebral cortex, whereas other thalamic nuclei provide scarce and diffuse inputs to the striatum but innervate more strongly the cerebral cortex (Fig. 5).

10.3. Function of the Thalamostriatal Systems

The exact role of the thalamostriatal pathway remains poorly understood. Kimura and his colleagues recently proposed that CM and PF supply striatal neurons with information that has attentional values, thus acting as detectors of behaviorally significant events occurring on the contralateral side. These observations are consistent with a positron emission tomographic study in humans showing activation of the CM/PF complex when participants switch from a relaxed awake state to an attention-demanding reaction-time task. Two main functional characteristics of CM/Pf neurons were disclosed in monkeys. First, CM/Pf neurons have multimodal properties; that is, they respond to a large variety of

sensory stimuli (auditory, visual, somatosensory) presented either in or outside sensorimotor conditioning tasks. Second, CM/Pf neurons are temporally tuned (i.e., they can generate in a timely fashioned manner discrete and coherent responses to a wide variety of sensory stimuli). On the basis of their latency and pattern of responses to sensory stimuli, CM/Pf neurons have been categorized into two main populations, namely those that display short-latency facilitatory responses (SLF neurons) or long-latency facilitatory responses (LLF) to sensory events. These two populations are largely segregated in the CM/Pf complex, SLF neurons being mainly found in Pf, whereas LLF are particularly abundant in CM. Responses of both types of neurons are not associated with reward. This contrasts them from the tonically active neurons (TANs; putative striatal cholinergic interneurons); one of their main targets in the striatum (discussed previously), which under the same experimental conditions, respond preferentially to rewarding stimuli. However, CM/Pf inputs are required for the expression of the sensory responses of TANs acquired through sensorimotor learning. Inactivation of CM/Pf decreases the characteristic pause and subsequent rebound facilitation—but does not affect the early short latency facilitation—of TANs in response to sensorimotor conditioning. Taking into consideration the importance of the dopaminergic system in modulating striatal activity through TANs, one may suggest that the behaviorally sensory events transmitted along the thalamostriatal projections from CM/Pf, in coordination with the motivational value of the dopamine inputs, provide a strong basis for proper selection of actions through the basal ganglia thalamocortical/striatal circuitry (see Smith and Raju [2004] for review).

The information flowing along the thalamostriatal pathway from CM/Pf and its functional relevance for basal ganglia functions likely differ from the signals provided to the striatum by the thalamostriatal projections from the ventral motor nuclear group. Although the functions of the ventral thalamostriatal projection remains unclear, the possibility that this system may serve as a positive reinforcer of striatal neurons involved in performing a selected behavior should be considered.

11. SYMPTOMS AFTER THALAMIC LESIONS

As discussed previously, the thalamus is the main relay center for sensory information to the cerebral cortex. Because of the high degree of functional specificity, lesions of specific thalamic nuclei would be

expected to result in an impairment or loss of specific sensations in the opposite half of the body. However, because thalamic nuclei are relatively small and very close to each other, lesions that affect only one of them are very rare. Furthermore, the fact that large ascending and descending fiber bundles travel through the thalamus often makes thalamic lesions multisymptomatic and difficult to interpret. Tumors and especially vascular lesions related to the middle and posterior cerebral artery may involve the thalamus and induce various kinds of behavioral changes in cases where the entire thalamic area receiving somatosensory modalities is destroyed, deep sensibility and discriminating senses are severely impaired, whereas the sense of touch, temperature, and pain perception are less affected. A distinguishing feature of thalamic lesions is the appearance of spontaneous “burning” pains, often referred to as thalamic pain syndrome. The thalamic pains are usually very intense, frequently irradiate to the entire half of the body, and are usually intractable to analgesics. They are often present together with a sensory loss, although they may occur without this loss, and usually then as a initial symptom. No universally accepted explanation for these pains has been given. Some believe that they are the result of vasomotor disturbances in the thalamus, and others assume that disappearance of cortical inhibition or lack of intrathalamic association may be the cause. These pains usually occur with small vascular lesions and can be partly abolished by stereotactic thalamotomy (see later).

The thalamus, particularly the caudal intralaminar nuclei, is also the target of some neurodegenerative diseases such as Parkinson's disease. Some patients with multinutritional deficiencies resulting from starvation or alcoholism suffer from Wernicke-Korsakoff's syndrome, characterized by various motor and short-term memory problems. Autopsy often reveals neuronal degeneration of the anterior thalamic nuclei and mammillothalamic tract in these patients. Functional and pathologic changes have been found in the mediodorsal nucleus of schizophrenics. Imaging studies reported hypometabolism in medial thalamic regions of patients with schizophrenia, and morphologic studies have described a 30% to 40% cell loss in the parvocellular and densocellular regions of the mediodorsal nucleus of schizophrenics.

The thalamus may also be the target of stroke due to obstruction of arterial blood supply. Although such cerebrovascular accidents usually result in complex ill-defined symptomatology because of the variable extent of the lesion, four types of stroke

syndromes have been characterized after thalamic ischemia: (1) Ischemia of the paramedian thalamus in thalamic-subthalamic territory: These infarcts are usually bilateral and result in a wide variety of symptoms including alterations in consciousness, neuropsychological disturbances, and abnormalities or paralysis of vertical gaze. Disturbances of memory and personality changes are also common. Significant bilateral involvement of intralaminar nuclei may result in permanent coma or hypersomnia. These symptoms have been attributed to lesions of the intralaminar and mediodorsal nuclei. The gaze control problems are likely due to disruption of connections between the intralaminar nuclei and the oculomotor centers in the premotor cortex. Selective lesion of intralaminar nuclei results in varying degrees of inattention and neglect, which is consistent with enhanced positron emission tomography activity in these nuclei in tasks that require increased alertness and selective attention. In some patients with preferential left hemisphere lesions, language deficits are also noticeable likely due to thalamic disconnections of Broca's area. (2) Ischemia of the posterolateral part of the ventral nuclear group and internal capsule: Such a lesion, which may occur after infarct of the inferolateral arteries, results in the thalamic syndrome of Dejerine and Roussy characterized by contralateral hemiplegia and hemianesthesia accompanied with astereognosis and hemiataxia. These are likely due to functional changes and disconnections of the ventral lateral and ventral posterior nuclei. (3) Ischemia of posterior thalamus: Stroke of posterior choroidal arteries results in visual defects due to disturbances of blood flow to the lateral geniculate nucleus. Problems with saccadic and pursuit eye-movements as well as variable degrees of aphasia and amnesia may also be found in these patients if the infarct involves the pulvinar or laterodorsal nuclei. (4) Ischemia of anterior nuclei and related mammillothalamic tract: This region will be affected after stroke of polar arteries. It usually results in complex cognitive disturbances as well as apathy, lack of appetite, and memory dysfunctions. Amnesia is a common symptom that results from thalamic damage of the mammillothalamic tract, anterior nuclei, and mammillary bodies.

12. THALAMOTOMY AND THALAMIC DEEP BRAIN STIMULATION FOR BRAIN DISEASES

The first stereotaxic thalamotomies in humans targeted the mediodorsal nucleus for the treatment of psychiatric diseases. The ventral anterior and center

median nuclei were later found to be suitable lesion sites for the treatment of psychosis or compulsive and aggressive behavior, respectively. The CM/Pf and adjacent thalamic nuclei also became targets of choice for the alleviation of pain syndromes. Hassler and Riechert (1954) first reported the use of thalamic lesions as treatment for rigidity and tremor in Parkinson's disease. Thalamic surgeries have been the treatment of choice for many brain diseases until the introduction of novel and highly effective pharmacotherapies for both psychiatric and motor disorders in the late 1960s. Stereotaxic functional surgeries for brain diseases have been revived and are currently used worldwide for poorly responsive patients to drug therapy. The recent introduction of electrical deep brain stimulation (DBS) has had a significant impact in this field. The reversible and adjustable nature of DBS makes it safer and less invasive than ablative procedures. Despite the limited understanding of its mechanisms, DBS is a treatment of choice for Parkinson's disease, dystonia, and tremor. More recently, successful cases of DBS for neuropsychiatric disorders and Tourette's syndrome have been reported. In Parkinson's disease, the subthalamic nucleus and internal globus pallidus are considered as the best stimulation sites to alleviate bradykinesia and rigidity, and the thalamus is, by far, the most reliable target for the treatment of various forms of tremor. DBS at the border of the Vop and Vim usually results in significant reduction of both parkinsonian and essential tremors. In general, the side effects of Vim-DBS are mild, which makes it the treatment of choice for most kinds of tremor-related motor disorders. In addition to Vim, DBS in the caudal zona incerta region is also effective in alleviating tremor. The advantage of this area over the Vim in Parkinson's disease is the additional beneficial effect of Zona Incerta (ZI) stimulation on other symptoms of the disease.

Recently, the CM/Pf complex has been suggested as a potential DBS target for Parkinson's disease and Tourette's syndrome. Although the beneficial effects observed in the few cases reported so far have to be confirmed in a larger cohort of patients, CM/Pf stimulation in some Tourette patients results in a dramatic reduction of vocal and motor tics with the disappearance of the sensory urge often seen in Tourette patients.

13. CONCLUDING REMARKS

This chapter briefly reviews the main anatomic features of the thalamus and explores some of the basic functional characteristics of specific thalamic nuclei. The basic circuitry of the thalamus relies on

three major sets of neurons: the glutamatergic relay neurons that project to the cerebral cortex and two classes of GABAergic intrinsic neurons arising from the reticular nucleus and local interneurons. The afferents to the thalamus come from a variety of cortical and subcortical sources that provide modality-specific information to different sets of thalamic nuclei, which in turn convey this information to the cerebral cortex through parallel segregated channels. These parallel pathways are particularly evident for modality-specific nuclei. The reticular nucleus, in concert with the corticothalamic projections and brain-stem modulatory afferents from cholinergic and aminergic cell groups, play a major role in inducing oscillations in large ensembles of thalamic neurons that underlie changes in the conscious state. It is clear that the thalamus is not a passive relay of information to the cerebral cortex. Instead, it actively filters the flow of information according to patterns that may vary with the state of consciousness and attention. The thalamus is connected with the cerebral cortex and also provides major inputs to various subcortical structures related to limbic and motor functions. The thalamostriatal projection from intralaminar nuclei is particularly important in that regard. Although specific lesions of the thalamus are not common because of the size and close proximity of modality-specific subnuclei, loss of somatic sensibility, cognitive deficits, memory impairment, and motor problems have been described after thalamic lesions. Furthermore, the loss of neurons in caudal intralaminar nuclei of parkinsonians supports the involvement of the caudal intralaminar nuclear group in basal ganglia functions and motor control. Neurosurgical therapies directed at lesioning specific thalamic subnuclei are often used for thalamic pains and tremor.

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Charles J. Heckman and William Z. Rymer

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1. A BRIEF REVIEW OF MUSCLE FUNCTION

1.1. Introduction: Muscle as a Mechanical System

All motor commands from the central nervous system are expressed through changes in the magnitude of neural excitation of skeletal muscle. These changes in muscle excitation give rise to force generation and to motion, whose magnitude depends on the properties of muscle and the mechanical loads experienced by the muscle. It is therefore necessary to begin a description of the neural regulation of movement with a short description of the relevant mechanical properties of muscle, with particular emphasis on the dual roles of skeletal muscle as a force generator and as a mechanical impedance with both elastic (i.e., spring-like) and viscous (frictional) properties.

1.2. Muscle as a Force Generator

Skeletal muscle acts as a machine that transforms chemical energy, stored in the form of high-energy phosphate bonds in the molecule ATP, into mechanical energy; that is, to force or to motion. The means by which this transduction of energy takes place is

relatively well understood, however space does not permit a full exposition here. Nonetheless, a short description of the cellular basis for the mechanical actions of muscle is important to our understanding of movement regulation, because many of the characteristics of neural activity are related to the special stimulus requirements of muscle.

As shown in Fig. 1A, muscle is made up of muscle fibers, which are, in effect, the cells of the tissue. These muscle fibers contain slender fibrils, called myofibrils, which bear a striated pattern. (The fact that the striations are in register on different fibrils within the muscle fibers gives rise to the striated appearance of the skeletal muscle fiber under the light microscope.) The force-generating element of the myofibril is the sarcomere (Fig. 1B), which is defined as the segment of the myofibril between two adjacent thin dark lines, called the Z lines. It is this recurring structure that gives rise to the striated pattern of the fibril and ultimately to the striated pattern of the whole fiber. The sarcomere contains thick and thin filaments, which bear the chemical moieties responsible for force generation. The sarcomere structure is held together by the giant protein titan (also called connectin). Titan runs perpendicular to the Z line. The Z line and titan are not active force-producing structures, but they are essential in transmitting force generated by the interaction between thick and thin filaments.

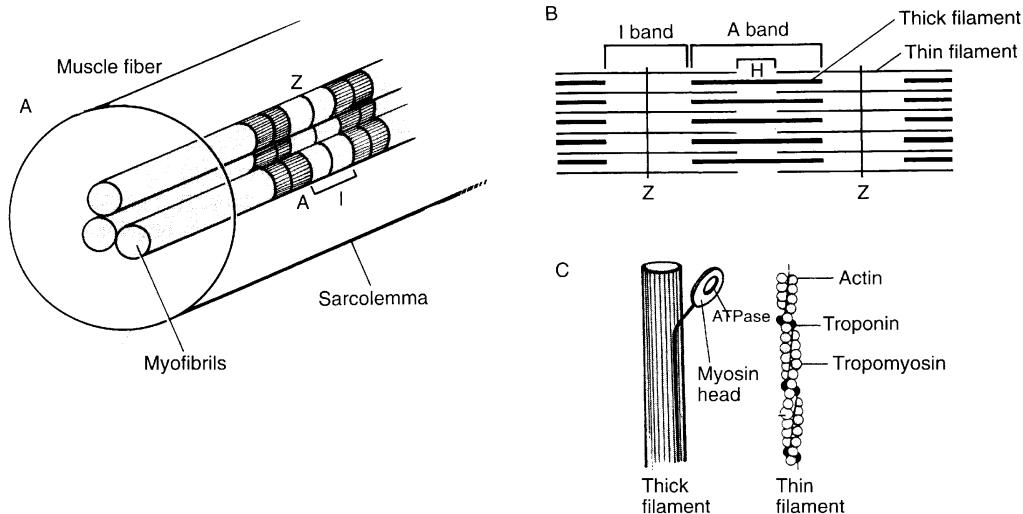


Fig. 1. (A) The constituent elements of the muscles include the muscle fiber, the membrane or sarcolemma, and the myofibrils, which appear striated because of the A and I bands, which have different optical properties. The myofibrils are the bundles of filaments that compose the sarcomeres, which are the segments between adjacent Z lines. (B) Components of the sarcomere: Each sarcomere contains thick and thin filaments. Thick filaments reside in the center of the sarcomere, and the thin filaments traverse the area from one sarcomere to another through the boundary, called the Z line. The thick filaments carry the myosin cross bridges, which interact with the thin filaments. The think-filament actin carries a receptor that binds the myosin cross bridge. (C) Molecular constituents of the sarcomere: The thick filament is composed primarily of myosin, which has a long tail and a protruding head forming the cross bridge. The head incorporates an ATPase binding location, which is needed for establishing the high-energy mechanical state of the cross bridge. The thin filament is made up of actin monomers, which are organized in a helical chain, forming F actin. Regulatory proteins lie in the grooves of the F actin strands. These are myosin, an elongated molecule that lies in each of the two grooves of the actin helix, and troponin, a peptide that occurs at periodic locations along the thin filament.

The thick filament is made up primarily of myosin, which is a long-tailed molecule with a globular head and a flexible neck. The myosin molecule is made up of two heavy chains, which form the body of the molecule, and four light chains, which are sequestered in the head of the myosin molecule. The head contains two regions: the regulatory domain with the light chains and the catalytic domain for hydrolysis of ATP to ADP + Pi to produce energy. Different types of muscle tissue (skeletal, cardiac, smooth), muscle at different stages of development (e.g., embryonic and neonatal), and muscles from different animal species show differences in the myosin heavy chain isoforms. The myosin molecules are laid out in the thick filament so that their tails are packed in parallel, oriented along the long axis of the filament, and the heads protrude at regular spatial intervals around the circumference of the thick filament (Fig. 1C).

As shown in Fig. 1C, the myosin head is a key locus at which the chemical to mechanical transduction takes place. This transduction requires binding of the myosin head to receptor sites on the thin filament, and there are subsequent conformational changes in

the cross bridge, which are instrumental in producing muscle contraction (see further description later). The myosin head is also the locus for an important ATPase, which facilitates the process of muscle contraction.

The thin filament has a more complex structure in that it is composed of several different proteins. The major protein, actin, is a globular monomer that is packed in the form of a twisted chain, called F actin (Fig. 1C). The thin filament also contains so-called regulatory proteins, including *tropomyosin*, an elongated, rod-shaped molecule lying along the actin molecular helix, and *troponin*, which appears as a globular molecule, placed at periodic intervals along the thin filament. The troponin consists of various subunits, including Tn-C, which binds calcium, 4 Ca²⁺ on each subunit; TnI, which is a major inhibitory subunit; and TnT, which binds to troponin. These two proteins regulate contraction by controlling access of the myosin head to the actin receptor sites on the thin filament. The process of force generation takes place when myosin heads bind to exposed actin receptor sites on the thin filament.

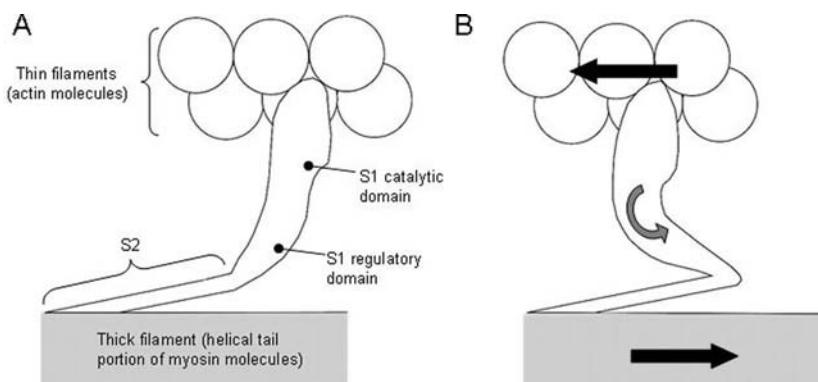


Fig. 2. The molecular mechanism of contraction. (A) The head of the myosin molecule, known as the S1 region, is bound to an active site on actin. The S1 head has two regions, the catalytic regions in which ATP is hydrolyzed to ADP + Pi, to produce energy, and the regulatory region, where the conformational change that produces force occurs. The S2 portion of the myosin molecule forms the link between the head and the helical tail of the molecule, which, along with many other myosins, forms the thick filament. Not shown is the fact that the S1 portion actually has a pair of heads, as the reason for this pairing is not yet clear. (B) When the reaction products of the hydrolysis of ATP to ADP + Pi are released, the resulting conformational change in the regulatory region of S1 occurs, causing the filaments to slide with respect to each other. This is the essence of the molecular motor that produces muscle force and changes in muscle length.

1.2.1. CROSS BRIDGES GENERATE FORCE AND MOVEMENT

It has long been appreciated that the thick and thin filament slide past each other in the process of movement. The molecular motor for movement and force is the cross bridge formed by the myosin molecule when it attaches to actin. The conformational change to generate force occurs within the cross-bridge head, which is known as the “lever arm” hypothesis (Fig. 2). This conformation change occurs when the reaction products ADP and Pi are released. Binding of a new ATP molecule allows the cross-bridge head to detach. Because of the structure of the sarcomere, with cross-bridge heads extending with different polarities in the two halves of the structure, force is generated toward the center. Thus muscles tend to exert tension (i.e., pull).

The process of cross-bridge binding and detachment can occur repeatedly within a given contraction cycle, so that a given cross bridge may bind, undergo conformational change, detach, and then rebind to a different actin receptor site. This process can continue as long as the level of free calcium ion (Ca^{2+}) in the sarcoplasm remains high (see further later) and as long as ATP synthesis keeps pace with the need for high-energy phosphate.

1.3. How Is Muscle Contraction Initiated?

1.3.1. THE ROLE OF FREE INTRACELLULAR CALCIUM IONS IN EXCITATION CONTRACTION COUPLING

Calcium ions (Ca^{2+}) are widely believed to regulate many cellular processes, including cell motility,

secretion, and synaptic transmission. Calcium also appears to be important for muscle contraction in at least two ways. Initially, increases in the concentration of calcium ions (Ca^{2+}) appear to control the sequence of events of contraction directly, by regulating myosin interaction with actin. Subsequently, calcium ion reductions act to terminate contraction.

The processes of excitation-contraction coupling rely on specialized structures called the T tubule and the sarcoplasmic reticulum (Fig. 3A, B). The T tubule, or transverse tubule, is a tube that communicates with the muscle fiber membrane surface and extends into the cell interior. It is capable of supporting action potential propagation well into the cell interior. The sarcoplasmic reticulum (SR) is a closed set of tubules and cisterns, which does not communicate with the cell surface, but which approaches the T tubule, forming specialized structures called triads. Depolarization of the T-tubule activates voltage-sensitive Ca^{2+} channels, which are coupled to ryanodine receptors linked to the SR. Ryanodine receptors then cause release of Ca^{2+} from the SR.

Under normal resting conditions, the concentration of free Ca^{2+} ions in the sarcoplasm is held at barely detectable levels ($<10^{-12} \text{ M}$). In response to neurally initiated depolarization of the sarcolemma, calcium release develops very swiftly, reaching concentrations of 10^{-7} M or higher in some types of muscle cells. The effect of these calcium ion increases is to change the conformation of the regulatory proteins on the thin filament, allowing nearby myosin cross bridges to access and to bind to exposed actin receptor sites.

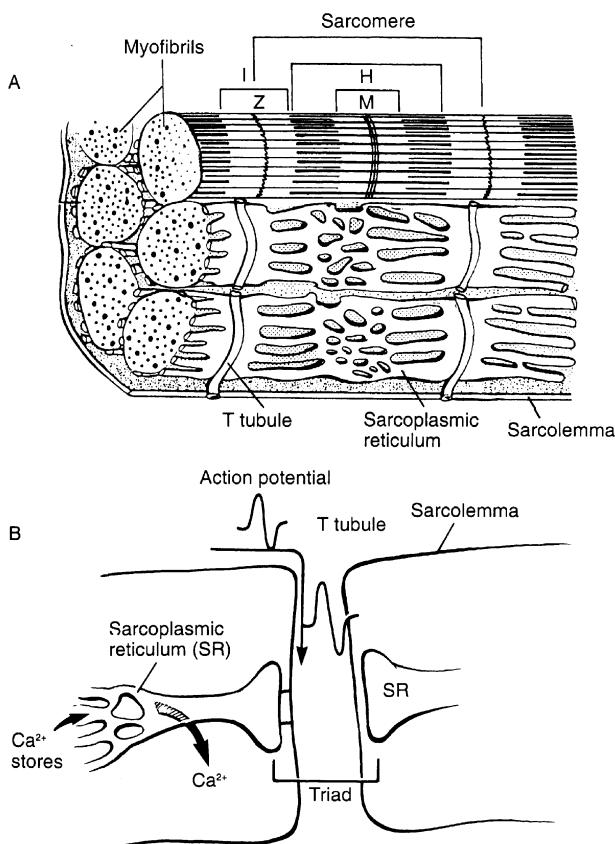


Fig. 3. (A) Components responsible for excitation-contraction coupling within the muscle contraction coupling. The muscle contraction is initiated by Ca^{2+} release from stores within the sarcoplasmic reticulum (SR). An action potential, initiated in the end-plate region, traverses the sarcolemma and is propagated inward toward the fiber center by conduction of the action potential into the T tubule. **(B)** The structures responsible for excitation-contraction coupling are seen in more detail. The terminal cisternae of the sarcoplasmic reticulum are arranged symmetrically around the sarcolemma of the T tubule, forming a triad. Depolarization of the plasma membrane of the muscle cell induces calcium release from the adjacent sarcoplasmic reticulum by chemical or voltage signals.

1.4. How Does Muscle Contraction End?

Because an increase in calcium ion concentration is the primary event that initiates contraction, it is reasonable to suppose that calcium ion reduction acts to terminate contraction, and this does appear to be the case. When calcium release is terminated, governed by the recovery of the muscle fiber membrane potential to normal levels, calcium is reabsorbed swiftly by active transport from the sarcoplasm into the sarcoplasmic reticulum. This reabsorption process is energy dependent, because the calcium has to be reabsorbed against a substantial concentration gradient. One molecule of ATP is degraded for two molecules of calcium absorbed.

1.5. Energetics of Muscle Contraction: Specialization of Muscles Fibers

ATP is used to promote cross-bridge detachment and conformational change in cross bridges and also to support active transport of calcium into the SR. ATP therefore plays a pivotal role in many processes that are responsible for muscle contraction. ATP is generated as a result of several biochemical processes in the muscle fiber, including degradation of free fatty acids (that are absorbed from the bloodstream) and/or degradation of glucose. This glucose may also originate directly from capillary absorption, or it may be generated by the degradation of intracellular glycogen stores. The breakdown of glucose or free fatty acids (FFAs) can then proceed using the machinery of either oxidative metabolism or of glycolytic metabolism when oxygen-dependent mechanisms are less readily available.

1.5.1. RELEVANCE OF METABOLIC SPECIALIZATION TO SPECIALIZATION OF MUSCLE FIBERS

Fibers in different skeletal muscle, and even fibers in the same muscle, often show differences in metabolic properties to allow functional specialization of mechanical muscle performance. In humans, muscle fibers are subdivided into three groups, types I, IIa, and IIx. These different fiber types are specialized in terms of speed of contraction and the degree of muscle fatigability (see Section 5.4).

IIa and IIx muscle fibers are fast twitch and contain very high glycogen concentrations, and these appear to rely primarily on glycolytic pathways to generate the ATP necessary for contraction. Glucose is released from stored glycogen by a phosphorylase enzyme and then is degraded to lactate, generating a few molecules of ATP per molecule of glucose degraded. ATP may also be generated on a short-term basis by transfer of high-energy phosphate ($\sim\text{P}$) from s storage location on creatine. Type I fibers are slow twitch and rely on oxidative metabolism, which is a far more efficient means to generate the high-energy phosphate of ATP. These fibers typically use absorbed glucose or free fatty acids and oxygen and have highly specialized metabolic apparatus for oxidative phosphorylation, including large numbers of mitochondria, substantial intracellular myoglobin (which is binding compound, rather like hemoglobin, which facilitates oxygen storage), and relatively modest or absent glycogen stores. These latter (oxidative type) fibers are usually surrounded by a dense capillary network, presumably to facilitate oxygen and FFA delivery to fibers. Type I fibers have much greater fatigability resistance than IIa and IIx fibers. (These

issues will be revisited when we describe the use of functional groups of muscle fibers, called the *motor units*, in the regulation of muscle force generation.)

2. MECHANICAL PROPERTIES OF WHOLE MUSCLE

Neurally activated muscle has several important mechanical properties, which are instrumental in the performance of movement.

2.1. Muscle Behaves Like a Spring

When an active muscle is stretched, muscle force output increases progressively with increasing muscle length. This proportional relation between force and length is the defining property of a spring. As shown in Fig. 4A, for a given level of neural

excitation, the force increases with increasing muscle extension, until a maximum is reached. Beyond this optimum length, muscle force begins to decline. This is the length-tension relationship. The position of the length-tension in relation to joint excursion remains unknown in most muscles, though in anti-gravity muscles acting at the ankle, the optimal length is near the physiologic maximum allowed by the range of joint rotation. The form of the length tension relation also varies somewhat with the rate of neural excitation. As stimulus rate decreases, the length at which the force begins to increase steeply increases and the optimal length increases. The reasons for these length-dependent changes in force are not entirely clear, but changes in length-related increases in calcium release from the sarcoplasmic reticulum are likely a significant factor.

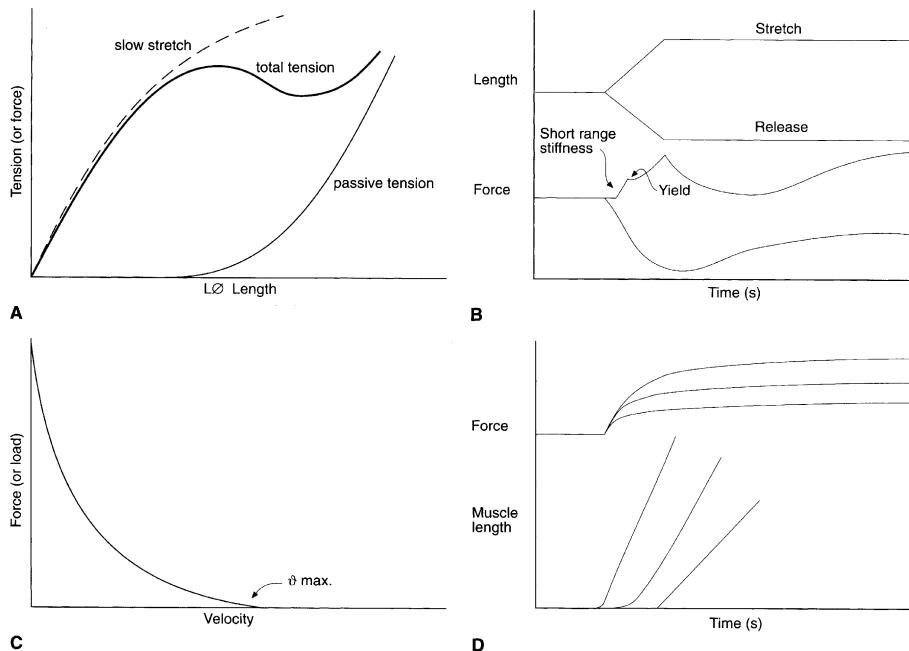


Fig. 4. Mechanical properties of skeletal muscle. **(A)** Length of tension properties: The isometric length-tension relation is derived by stimulating the muscle nerve at a constant intensity and frequency while holding the muscle rigidly at a designated length. If this length is progressively increased, muscle force increases progressively until reaching a maximal value at the maximal physiologic length in the body (L_0). If extension is continued beyond this maximum, the muscle force begins to fall, before ultimately increasing again at extreme lengths. This decline and subsequent increase in force at longer isometric lengths is attributable to the contribution of passive tissues surrounding the fibers. **(B)** Mechanical properties of active muscle subjected to symmetric stretch and release: When electrically stimulated muscle or deafferented active muscle is subjected to symmetric stretch and release of comparable velocity and amplitude, the force responses are grossly asymmetric. During stretch, the muscle initially has a region of elevated stiffness called the short-range stiffness, which is followed by an abrupt decline in stiffness, called the yield. At the end of the stretch, there is a secondary decline in force, which gradually recovers, with the muscle reaching a steady state only after several hundred milliseconds. During shortening, there is a relatively smooth decline in force, which settles to an isometric value. **(C)** Force-velocity relations: When muscle is allowed to shorten against a constant load, the magnitude of the shortening velocity, when measured at a constant length, is related to the load magnitude as a hyperbolic function. **(D)** Correlations between load and shortening velocity: If muscle force is generated with the muscle attached to the load, no shortening can occur until muscle force exceeds the load magnitude. When the load is minimal, this level is reached quickly, and muscle shortening begins relatively early and takes place at a relatively high velocity. When the load is maximal, the shortening begins after a longer period and takes place at a relatively slow velocity.

This spring-like description of muscle mechanics is broadly accurate for *isometric* length conditions (in which the length of the muscle is clamped at each measurement point), but it becomes even more precise for slow stretches, which generate a near-linear force-length relation, resembling a simple spring even more closely. The significance of these spring-like responses will become more clear after we describe the added effects of reflex action, however one clear benefit of these spring-like properties is that muscle forms a compliant interface with the external world and thus acts somewhat like a shock absorber.

2.1.1. DEVIATIONS FROM SPRING-LIKE BEHAVIOR

If active muscle devoid of reflex control is stretched rapidly from an initial isometric state, then the aforementioned spring-like behavior is disrupted, and muscle stiffness can be seen to change sharply with increasing stretch. As shown in Fig. 4B, once the stretch exceeds a fraction of a millimeter (typically 300 to 400 μm), the initial steep rise in force is interrupted, and muscle force declines sharply, sometimes even falling below the initial prestretch level. The initial high stiffness region is called the *short-range stiffness* and the subsequent sharp decline in force the *muscle yield*. Although this short-range stiffness and yield are most distinct in slow-twitch muscles (such as the soleus), there is routinely a change in stiffness during stretch even in fast-twitch muscle, once the length change exceeds a fraction of a millimeter. The initial high stiffness is attributable to the stiffness of a population of attached myosin cross bridges, and the steep decline in force (and in stiffness) is a result of stretch-induced cross-bridge rupture.

2.1.2. ASYMMETRY OF MUSCLE MECHANICAL RESPONSE TO STRETCH AND RELEASE

There is also a profound asymmetry of the force response to symmetrical stretch and release, which represents a substantial deviation from classic spring-like behavior. Although the force changes after a few hundred micrometers of stretch or release are usually symmetrical, the two responses then depart substantially from this pattern. Specifically, muscle remains quite stiff during release, but as described above, it often shows a substantial decline in overall stiffness during stretch.

These mechanical characteristics represent a significant departure from spring-like behavior of muscle, and they pose substantial difficulties for any neural control mechanism, because the force changes develop so quickly, and because they are so profound.

2.1.2.1. Force-Velocity Relations. Although muscle shows spring-like behavior for both slow extensions and slow shortening, the magnitude of the force generated during shortening of muscle is determined primarily by the speed with which the shortening occurs. Conversely, there is a well-defined relation between the speed with which a muscle can shorten and the load that it can carry. For example, when a muscle is activated electrically and allowed to shorten against a load, a characteristic sequence of length and force changes has been observed, in which the shortening speed declines with increasing load magnitude.

A typical shortening experiment is diagrammed in Fig. 4C, D. Initially, as the muscle force develops, it may not be sufficient to overcome the opposing load, and no motion takes place. (This is the *isometric* phase, in which force is increasing without accompanying reduction in muscle length.) Once the force generated exceeds the magnitude of the opposing load, the muscle begins to shorten progressively at a constant velocity. The shortening velocity is very rapid when loads are small and declines when the applied load is increased. Ultimately, shortening velocities become very slow when loads are very large, when measured with respect to the maximum force-generating capacity of the muscle.

The form of the relation between shortening velocity and applied load has been studied extensively and is well characterized. Figure 4C illustrates a typical force-velocity relation drawn from mammalian skeletal muscle, showing that the decline in force, (relative to the isometric state) is very steep, even at modest shortening velocities, indicating that the effect of movement on muscle force generation is quite profound.

Although the classic force-velocity relations were described using muscle shortening against controlled loads, the converse relation also applies, in that the maximum force generated falls steeply when muscle *velocity* is regulated by the experimenter. Although these force-velocity relations may seem to be somewhat arcane, they are very important in regulating the speed of human movement, and they are ultimately limiting to motor performance.

2.2. Neural Excitation of Muscle

At the beginning of this chapter, we described the sequence of events lying between the neural excitation of muscle and the resulting force generation. We will now describe the relations between the frequency of neural excitation and the resulting muscle force.

2.2.1. FORCE-FREQUENCY RELATIONS

When the motor axon is electrically activated by a single short pulse, or naturally by synaptic excitation of the motoneuron, a single action potential is transmitted from nerve to muscle, producing a transient increase in muscle force, described as the muscle *twitch*. There is a substantial delay between the arrival of the excitatory potential in the muscle, and the beginning of muscle force generation. This delay is described as the *excitation contraction* delay, and it may reach 3 to 5 ms or more, depending on the type of muscle being examined. In all mammalian skeletal muscles, however, the twitch has a characteristic form, in which there is a relatively rapid rise from onset to peak force and then a more gradual decay. This time to peak force and time to half peak force during the declining phase vary greatly in different types of muscles, but the twitch is routinely asymmetric, with a more prolonged declining phase.

As shown in Fig. 5A, when muscle is activated repeatedly by a train of action potentials, the mean force level generated varies greatly with the rate of neural activation. When the excitation rate is sufficiently low, so that the force generated by the twitch has returned to baseline between each twitch, there is no net force increase, and the maximum force reached is simply that generated at the peak of each individual twitch. However, as the rate of stimulation is increased, the new nerve impulse arrives before the force generated by the previous twitch has completely

dissipated. There is then force summation, and successive twitches generate a progressively increasing force level. This force level increases over the first few impulses, then reaches a plateau level that remains relatively constant for some seconds. When the individual twitches are still discernible, this plateau in force is described as a *partially fused tetanus*. When no individual twitch transients are evident, and the force trace is smooth, the response is described as a *fused tetanus*.

The practical implications of this force-frequency relationship are twofold. First, as shown in Fig. 5B, there is a nonlinear relationship between stimulus rate at which motor axons are activated and the resulting mean muscle force, although the effects of such rate increases vary in different kinds of muscle. This relation is sigmoidal in shape. The example illustrated in Fig. 5 shows that at very low rates (usually between 3 and 6 impulses per second), significant relative increases in rate produce very little increase in mean force. However, after rates of 8 to 10 impulses per second are reached, increases in rate produce substantial increases in mean force, and this high sensitivity to increasing rate continues until relatively high rates are achieved. For one of the muscles illustrated in the figure, the rates may ultimately reach 30 or 40 pulses per second, but this rate varies widely in different kinds of muscles.

This nonlinear relation between rate and force is extremely important, because it requires that the

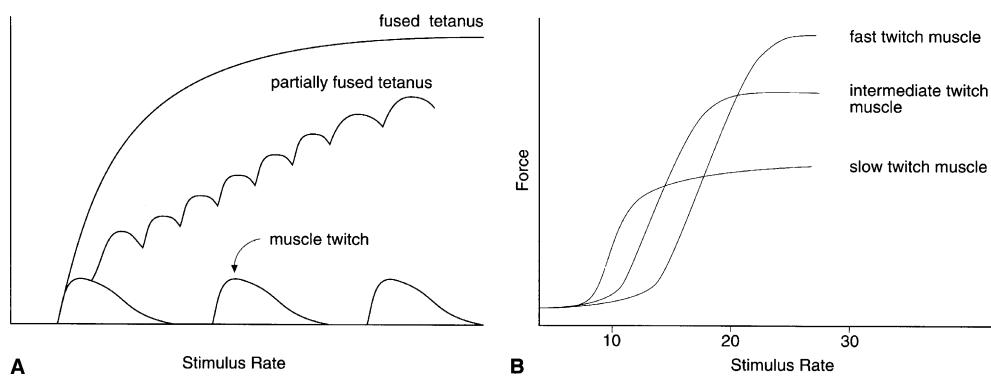


Fig. 5. Relationship between stimulus rate and muscle force. (A) Effect of stimulus frequency on muscle force output. When muscle is stimulated with a single, short stimulus to the muscle nerve, the resulting force change is described as a muscle *twitch* in which the force rises quickly to a maximum and then decays slowly. If stimuli are applied at low rates, there is no net force accumulation. If the stimulus rate is increased so that the mean force level accumulates, the force rises progressively, and it is described as a *tetanus*. If the individual twitch transients are still visible, the tetanus is called an *unfused tetanus*, and if the force trace is entirely smooth, the tetanus is described as a *fused tetanus*. (B) Force rate relations for different muscles: When a slowly contracting muscle, such as the soleus, is stimulated at different rates, the force begins to rise at relatively low rates, and the plot is a sigmoidal curve, demonstrating a maximal force arising at 20 to 30 pulses per second. For a fast-twitch muscle, the steep portion of the sigmoidal curve is moved significantly to the right of the plot and may not be reached until rates of more than 10 pulses per second are applied. Maximal force values may not be reached until rates exceed 50 or 60 pulses per second.

motor units must be activated at particular rates in order for muscle to be an optimally effective force generator. In most instances, the nervous system generates motoneuron discharge rates that do indeed lie within the steeply rising portion of the sigmoidal relationship for each motor unit. However, whereas the form of the rate-force relation is routinely sigmoidal, the exact magnitude and shape of the sigmoidal curve varies greatly for different types of muscle fibers. Slowly contracting muscle fibers reach the steep portion of their sigmoidal curve at relatively low discharge rates, whereas rapidly contracting fibers need more frequent neural activation to achieve a full tetanus (see Section 5.4). As a consequence, in rapidly contracting muscles, the sigmoidal relation is moved to the right. In addition, the form of the force-rate relations will change for muscle fibers as they become fatigued, or as they are activated repeatedly, and achieve a state called *potentiation*, in which twitch size increases during repetitive activation.

The means by which the motoneuron rate is tuned to match the contractile properties of the associated muscle fibers is not entirely clear, although this “matching” must also be achieved acutely to accommodate the changing contractile properties of muscle fibers during repetitive activation. For example, in fatigue, the muscle fiber contraction time and relaxation times slow substantially, meaning that lower motoneuron discharge rates are required to achieve maximal force. On the other hand, when other fibers are activated repeatedly, twitch size may increase, and contraction times may change because of a phenomenon called *potentiation*.

3. MUSCLE RECEPTORS

3.1. Muscle Receptors: What Is Transduced?

3.1.1. INTRODUCTION

Skeletal muscle contains several types of specialized receptors, whose properties, structure, and functional contributions to movement regulation are now relatively well understood. Figure 6 illustrates the three main classes of muscle receptors in muscle, namely muscle spindle receptors, Golgi tendon organs, and free nerve endings.

3.1.2. MUSCLE SPINDLE RECEPTORS

As shown in Fig. 6A, the muscle spindle consists of a cluster of slender muscle fibers, called *intrafusal* fibers, contained within a fluid-filled capsule. The muscle spindle lies adjacent to other regular muscle fibers and traverses the length of the muscle (or a

substantial fraction of it), from the tendon of origin to the tendon of insertion. Because of this arrangement, the muscle spindle is said to lie *in parallel* with regular skeletal muscle fibers. Under isometric conditions, active muscle force increases, such as those mediated by neural excitation of muscle fibers, will elongate series elastic elements, including the tendon, and reduce tension on the spindle and on the spindle receptors, causing a *reduction in afferent discharge rate*. This force-induced reduction of discharge is called *unloading*, and it is often used as a test to distinguish muscle spindle receptors from the in-series receptors, the tendon organ, which increases its discharge during active muscle force increase.

The muscle spindle carries two kinds of specialized sensory terminals and a dense and highly specialized efferent innervation. The structure of these receptor terminals is shown in Fig. 6B. There is a large, typically annulospiral shaped ending, wrapped around the central portion of all intrafusal fibers in the spindle, and a smaller, often branching sensory terminal, which is located more peripherally, toward the polar regions of the spindle. Based on their differing responses to muscle length increase, the first is called the *primary* ending and the other the *secondary* ending.

Intrafusal fibers display specialized anatomic features, which separate them into two broad categories. A small fraction of the intrafusal fibers (usually only one to two in each spindle) show clusters of nuclei in the central or *equatorial* region of the fiber and are called *nuclear bag* fibers. The majority of the intrafusal fibers are slender and elongated, with nuclei arranged in chains. These are labeled as *nuclear chain* intrafusal fibers. We now know that there are further subspecializations of these intrafusal fibers (bag_1 , bag_2 , long and short chain, etc.), but these distinctions are not vital for our understanding of spindle receptor function at the present time.

The behavior of the muscle spindle receptor appears to be governed primarily by the mechanical properties of the supporting intrafusal muscle fiber, although the intrinsic biophysical properties of the primary and secondary receptor terminal areas may also be somewhat different. Nuclear bag fibers have little or no contractile material present in the equatorial regions where the nuclei are located. Instead, the contractile regions are confined to the intrafusal fiber poles. This has two consequences. First, if intrafusal fibers are stretched, the mechanical resistance exerted by the pole is likely to be different from that of the equatorial region, even at rest. Because the poles are more viscous in character, more rapid stretches would extend the equatorial regions

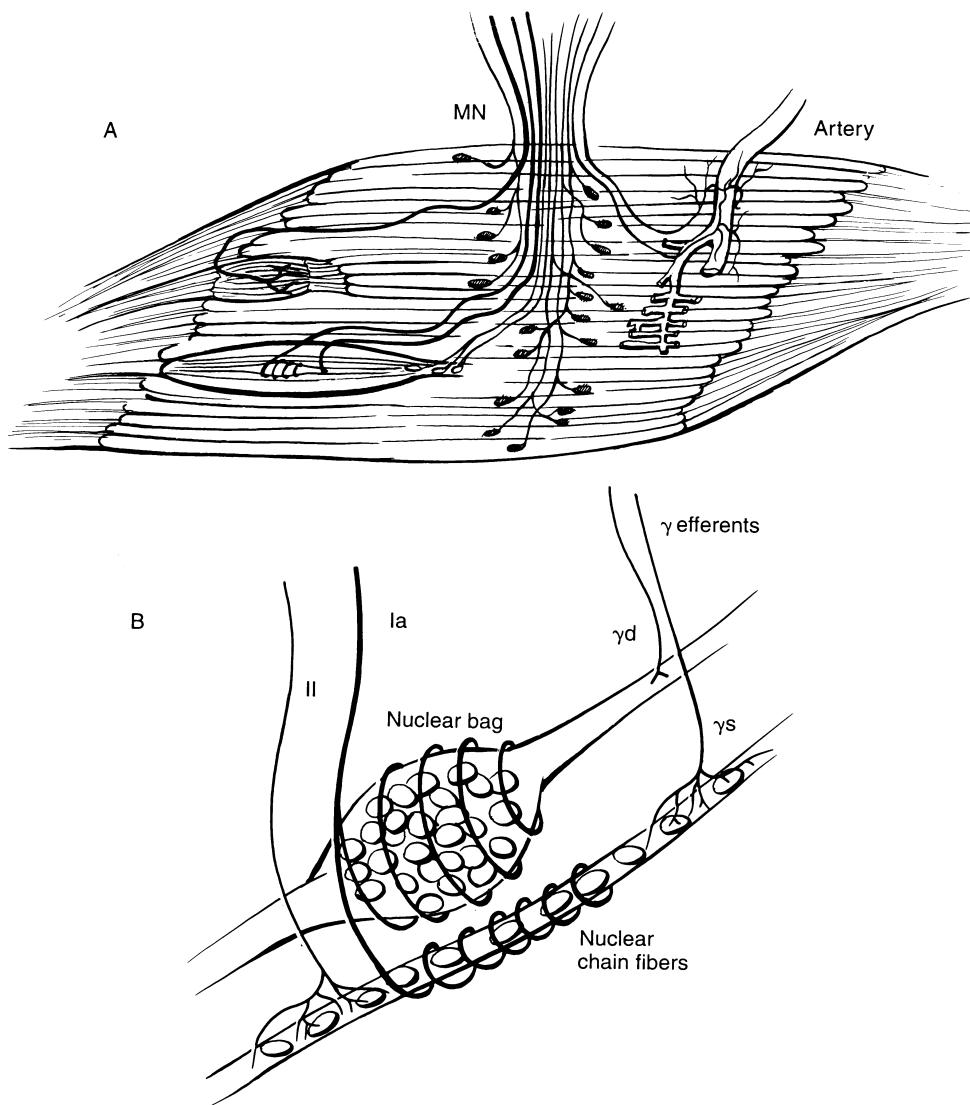


Fig. 6. Organization of muscle receptors: (A) Extrafusal muscle, with fibers reaching from tendon at one end to tendon of the other end. The motor innervation terminates as a motor point in which end-plates are distributed across the muscle. Two of the key encapsulated muscle receptor organs are the muscle spindle and the tendon organ. The muscle spindle consists of an encapsulated structure in which small fibers, the intrafusal fibers, reach from tendon on one end to tendon on the other. Around the central portion of the spindle, there is a receptor terminal with an annulospiral structure called the primary ending. In the polar regions of the spindle, there is a separate spindle innervation, called the fusimotor innervation. (B) Expanded view of the intrafusal fibers and receptor terminals of the muscle spindle. The spindle contains large nuclear bag fibers, which are characterized by a cluster of nuclei in the central or equatorial region. Other intrafusal fibers, called nuclear chain fibers, have the nuclei arranged serially. The primary ending usually has an annulospiral appearance, in which the receptor is coiled around all of the intrafusal fibers. The secondary ending has a branched or coiled structure and is located more peripherally. Gamma efferent innervation (γ) is illustrated with gamma plates on the bag fiber and branching terminals on the chain.

disproportionately, because the poles would be more resistant to stretch under these conditions. Because the primary ending terminals are located largely around the equatorial regions, more rapid muscle stretches will impact the receptor terminals to a greater extent.

A second way in which intrafusal structure affects spindle receptor behavior is that efferent spindle innervation, which activates the polar regions of the muscle

spindle, will produce increased force and shortening of the polar regions at the expense of the more elastic equatorial zone. This is especially likely in the bag fibers, which appear to have the greatest difference between the mechanical properties of poles and equator. Although the nuclear chain fiber shows less structural inhomogeneity, mechanical observations indicate that the polar regions may also be somewhat less stiff than the

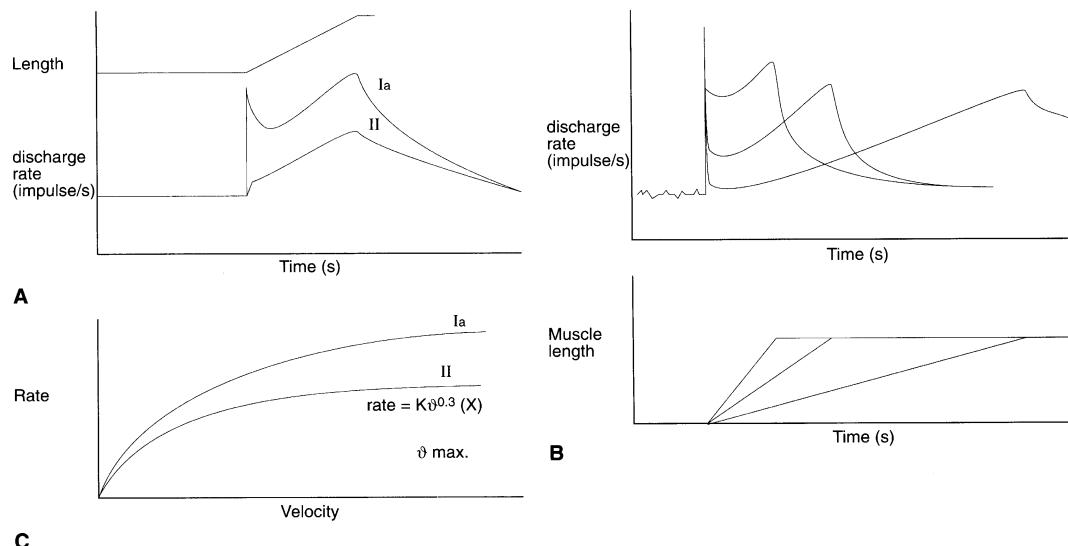


Fig. 7. (A) Responses of primary and secondary endings to constant-velocity stretch of a given amplitude: The primary ending shows a very steep step-like increment in the discharge rate at the beginning of the stretch, terminating in a brief cluster of action potentials, emitted at a very high frequency. This is called the initial burst. The rate increases progressively with increasing length and then falls steeply at the end of the ramp of the plotted curve, returning to a substantially lower level. The secondary ending has a much smaller incremental increase at the beginning of the ramp of the curve and may have a limited dynamic overshoot. (B) Velocity dependence of the primary ending discharge rate: Three velocities are illustrated, but the discharge rate increment changes modestly. (C) The rate increment changes modestly with increasing velocity. A 100-fold increase in stretch velocity produces a 2-fold to 3-fold increase in the discharge rate. The form of this correlation is that of a power function, with the velocity exponent ranging between 0.2 and 0.3. The graph also demonstrates that primary and secondary endings are scaled versions of each other.

equatorial zones in these fibers as well. These differences in the structural and mechanical properties of the polar region may help to explain the different responses of primary and secondary endings to muscle stretch.

Figure 7 illustrates the different responses of primary and secondary spindle receptor afferent fibers to constant velocity stretches of the receptor bearing muscle, beginning at a relatively short length. The primary spindle afferent shows a substantial increase of discharge during the dynamic phase of stretch, and this discharge rate drops to a much lower level when a new constant length is achieved. This appearance is broadly comparable with that of a velocity sensor, which would be expected to increase its output substantially during the dynamic phase of stretch, where the velocity reaches a constant level, and to drop the output substantially when velocity falls to zero. In contrast, the secondary spindle afferent is much less influenced by the speed of the stretch and appears to follow the length changes more closely, displaying relatively little dynamic overshoot during the ramp stretch.

When characterized in this fashion, it is possible to attribute predominant *velocity* sensitivity to muscle spindle primary receptors and predominant *length* sensitivity to the secondary endings. In fact, a more extensive comparison of the impact of different

stretch velocities on various receptor types as shown in Fig. 7B, C indicates that neither primary or secondary endings are especially sensitive to stretch velocity, as a 100-fold increase in stretch velocity induces only a 2- to 3-fold increase in discharge rate in either kind of receptor. Furthermore, the relative increase in discharge with increasing velocity is quite comparable between the two classes of receptors, presumably reflecting the similarities in mechanical properties of intrafusal fibers supporting both receptor types.

3.1.3. DEPENDENCE OF SPINDLE RECEPTOR AFFERENT DISCHARGE ON STRETCH AMPLITUDE

The response of the spindle receptor is also strongly influenced by the amplitude of stretch. Indeed, stretches of 100- to 200- μm amplitude produce disproportionately large increases in discharge rate, which would be unsustainable if continued over many millimeters of length change. This high sensitivity region is frequently called the *small-signal* region, and it is responsible for the so-called initial burst that is visible at the very beginning of a large-amplitude constant velocity stretch (Fig. 7A). This high-sensitivity small-signal response is also visible during other kinds of length stimuli, such as sinusoidal or square wave length change, and it is noteworthy in that the behavior of spindles in this region is essentially

linear, which means that spindle afferent discharge rate scales with increasing stretch amplitude and velocity.

3.1.4. EFFERENT INNERVATION OF THE MUSCLE SPINDLE

As outlined earlier, and illustrated in Fig. 8, all muscle spindles receive several types of efferent, or motor, innervation. This efferent innervation, which is broadly described as *fusimotor* innervation, arises from small motoneurons in the ventral horn, via small-diameter, slowly conducting myelinated fibers, called γ fibers. Alternatively, the efferent innervation may come from branches of regular, large-diameter skeletomotor fibers, displaying more rapid fiber conduction velocity. These larger fusimotor fibers are referred to as β , or *skeletofusimotor* fibers, reflecting the fact that they originate from branches of regular motoneuron efferents.

The actions of these two kinds of innervation (i.e., γ and β) on the muscle spindle are known to be broadly comparable and will not be discussed further here, although the relative use of these pathways is undoubtedly quite different, given their differing cellular origins and the likely difference in recruitment and rate behavior of the originating spinal neurons.

The effect of the fusimotor innervation on spindle afferent discharge depends essentially on the particular intrafusal fiber that is innervated. Those fusimotor efferents that innervate bag fibers enhance the dynamic responsiveness of the primary endings, because these endings are present on the bag fiber; hence they are described as γ_d (or β_d) dynamic fibers. On the other hand, fusimotor fibers that innervate nuclear chain fibers enhance both the length sensitivity

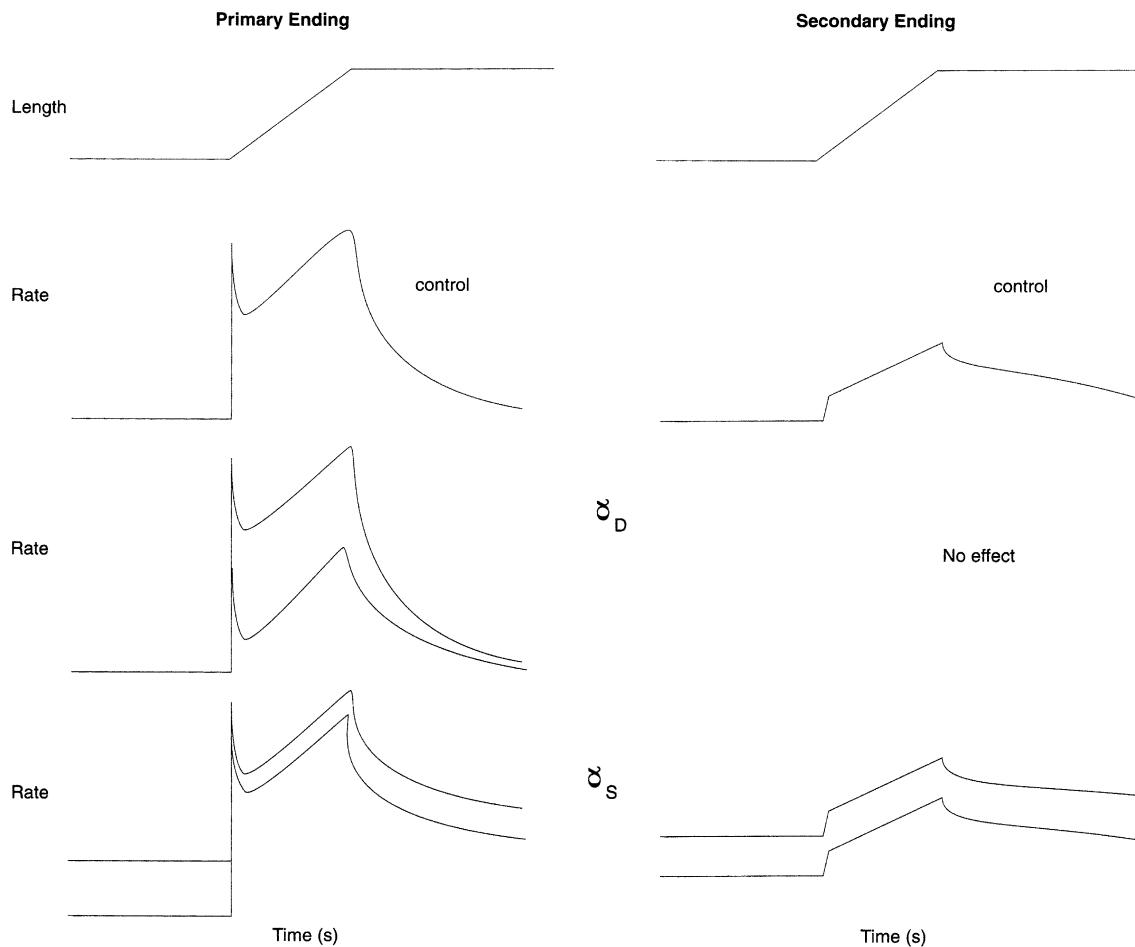


Fig. 8. Fusimotor effects on spindle afferents. This figure compares the effects of dynamic and static gamma motor neuron stimulation on primary and secondary muscle spindle afferents exposed to similar stretches. The second panel (from the top) on each side illustrates the control responses of primary and secondary endings, without added fusimotor input. Stimulation by gamma efferents of bag fibers produces a substantial increase in the dynamic response without a significant change in the initial and final firing rates. The dynamic motor neurons are called γ_d fibers. The efferent innervation of the chain fibers enhances the length sensitivity and discharge rate of both the primary and secondary endings. Because both initial and final or plateau rates are increased by gamma neuron stimulation, with little or no effect during the dynamic phase, this static efferent innervation is called γ_s .

and static background discharge rate of *both* primary and secondary endings. For this reason, they are described as static fusimotor (γ_s or β_s) fibers.

Figure 8 illustrates the different responses elicited by activating one or other class of fusimotor efferent fibers individually. (Of course, this situation is unlikely to take place in life, because both types of efferents are normally activated together.) Nonetheless, activation of individual γ_d fibers shows that the responses of spindle primary ending increases substantially, whereas γ_s activation produces changes mainly during the constant length, or isometric phase of muscle extension.

3.1.5. MUSCLE AFFERENT RECORDINGS IN INTACT SUBJECTS

In recent years, several methods allowing recording of afferent discharge in essentially intact, unanesthetized animal and human subjects have been developed. These include recordings from dorsal root afferents in cat using fine wire electrodes, dorsal root ganglion recordings in cat and monkey using sharp pin-like electrodes, and intraneuronal (or microneurographic) recordings in human subjects with insulated tungsten electrodes. The dorsal root and dorsal ganglion recordings in the cat have revealed the patterns of afferent discharge in a range of motor behaviors, including stance and locomotion, and the human studies have allowed accurate quantification of spindle afferent discharge during changing voluntary force and during voluntary movements of controlled velocity.

In many of the above studies, there has been shown to be a consistent pattern of spindle afferent excitation, in which motoneurons are activated broadly in concert with spindle afferent rate increases, suggesting that fusimotor activity increases with increasing skeletomotor activity. In many experiments in which α and γ discharge is recorded simultaneously, γ neurons appear to be largely activated before skeletomotor (i.e., α) activity begins, so that by the time extrafusal muscle fiber activation takes place, there has already been large-scale activation of γ fibers, giving rise to α - γ coactivation. At the present time, the rules governing the activation of fusimotor neurons are not entirely clear, although it has been demonstrated that fusimotor (i.e., γ) neurons may sometimes be activated without concurrent activation of α motoneurons. Whether there is the capacity to independently control fusimotor and skeletomotor (i.e., spinal) motoneurons remains to be verified.

Studies from intact human subjects have also been revealing with respect to afferent discharge during voluntary muscle contraction in either isometric contractions or during slow movements. Such studies,

which have been performed in muscles of both upper and lower limbs of human subjects, have the capacity to define the relation between fusimotor and skeletomotor activation more thoroughly than is possible in animal models, because of the voluntary cooperation provided by the human subjects. These human studies have shown that there is substantial variation in the level of fusimotor input during most naturally occurring movements, and that this fusimotor input is quite powerful in its effects on spindle afferent discharge.

As shown in Fig. 9, during voluntary isometric contraction, the discharge rate of muscle spindle afferents increases substantially, reaching a level

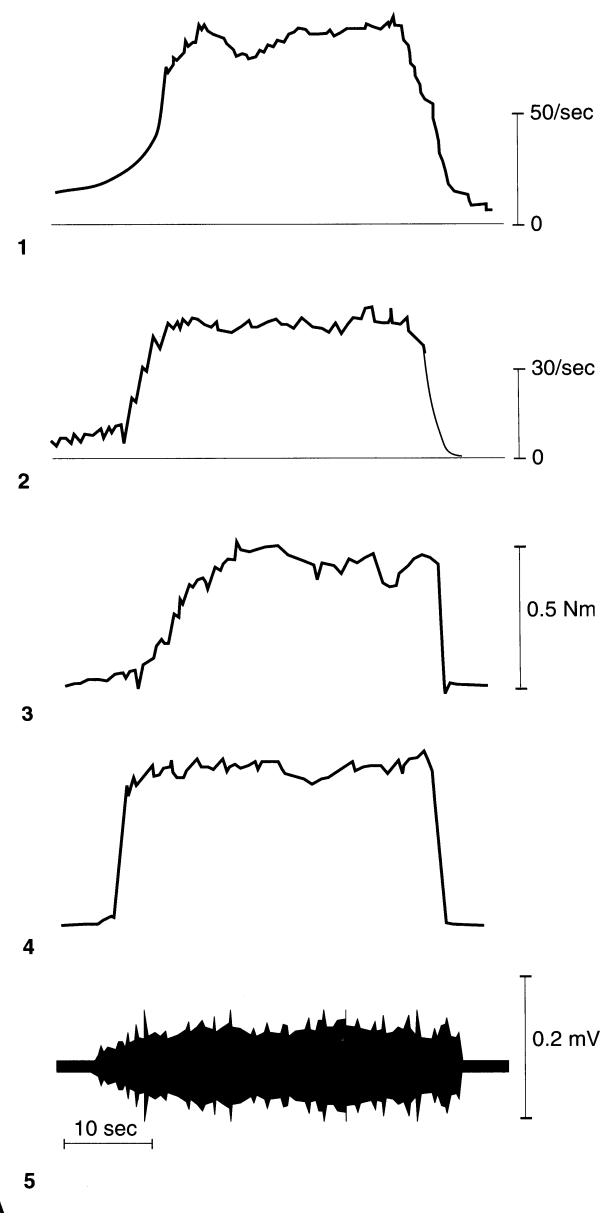


Fig. 9. (Continued)

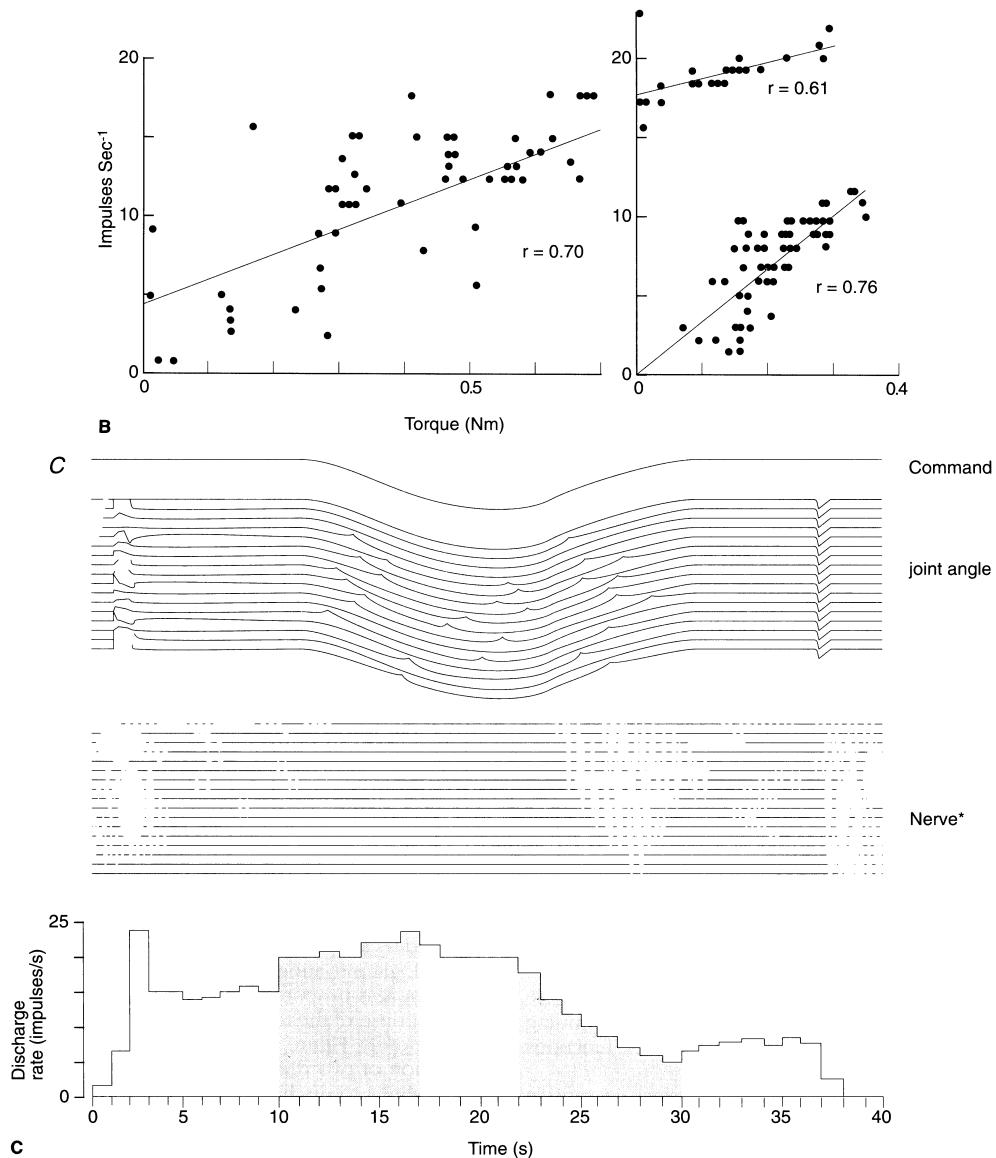


Fig. 9. Response of human muscle spindle afferents during voluntary contraction. **(A)** Response of a primary ending in a finger flexor during isometric contraction. Panel 1 shows the afferent firing rate; panels 2 through 4 show the torque increase; and panel 5 shows the associated electromyographic (EMG) response. The afferent rate increases steeply, even before there has been a significant increase in torque or EMG activity. The fact that the afferent rate increases sharply and is sustained even with progressive force increase indicates that a substantial increase in fusimotor activity must have taken place to offset tendon elongation and the associated internal shortening of the spindle. **(B)** Torque rate relations: The responses of one primary and two secondary endings illustrate that the discharge rate increases modestly but significantly with increasing isometric torque. **(C)** Response of muscle spindle afferent (nerve) from a finger flexor during a slow voluntary shortening. The visual target for movement is the command trace. The next series of traces (joint angle) depicts the sequence of actual movements. The plots labeled *nerve* are raster plots, in which the occurrence of an action potential appears as a dot. The lowest panel is a histogram, summarizing mean firing of the afferent during each phase of movement. Under conditions of slow voluntary motion, the fusimotor input to a muscle spindle may be sufficient to offset the muscle length change. In this sequence of voluntary movements, the recording of muscle spindle primary afferent responses is shown as a raster diagram. The response during the shortening and plateau phases is somewhat higher than that during the initial static or hold phase. However, the rate changes are modest, indicating the efferent innervation is able to compensate almost exactly for the change in muscle length.

much greater than that recorded at rest. In most of these studies, the impact of γ efferent innervation to the spindle is difficult to deduce, because increasing

force will induce elongation of tendon and other series of elastic components, allowing internal spindle shortening to occur. This elongation offsets fusimotor

action, at least in some degree. However, the finding (illustrated in Fig. 9A) that afferent discharge rate increases during force increase indicates that fusimotor input is more than able to offset the change in length series elastic elements. In addition, when muscle activation produces voluntary shortening, muscle spindle discharge rates may fall very little, provided that the rate of muscle shortening is modest (Fig. 9C).

Taken overall, these studies have shown that muscle primary spindle afferents are strongly activated during voluntary contraction, that this activation is very strong at the lowest force levels, and that it may then increase relatively little with increasing force. The effects of this fusimotor activation are such that it can compensate for muscle fiber shortening to a large degree, except when muscle shortening is rapid.

Finally, with respect to the type of fusimotor input activated in various movements, studies from both human and animal models also suggest that γ dynamic and γ static fusimotor activation takes place together, although the possibility that there may be independent activation of γ_d dynamic and γ_s static fibers under some naturally occurring conditions is not yet excluded.

3.1.6. SUMMARY OF SPINDLE BEHAVIOR

The mode of operation of fusimotor input to muscle spindles and its functional role is a matter of continuing debate, however it is likely that at least some of the following functions are fulfilled.

- (a) γ_s activation takes up the slack in muscle spindles, allowing both primary and secondary endings to respond sensitively to added small length changes. This raises the possibility that with the help of fusimotor input, spindle receptors are able to maintain a broad dynamic range, yet are still able to respond sensitively to small length perturbations.
- (b) Fusimotor innervation (either γ and/or β in type) may serve to match muscle spindles to the changing mechanical properties of muscle. For example, muscle becomes stiffer and more viscous as its level of activation increases. It is conceivable that efferent innervation to the spindle adjusts the mechanical properties of the intrafusal fibers to optimize the pattern of spindle response to compensate for these altered mechanical muscle properties.
- (c) γ_d input induces a substantial increase in the dynamic spindle response during muscle stretch but has much less effect during muscle shortening.

These effects may be important in providing appropriate asymmetry of reflex action in stretch and release of muscle (see later).

3.2. Golgi Tendon Organs as Force Transducers

3.2.1. STRUCTURE-FUNCTION RELATIONS OF THE TENDON ORGAN

As illustrated in Fig. 10A, the tendon organ is an encapsulated receptor, which consists of a branching nerve terminal, interwoven with collagen and elastic fibers lying between a group of muscle fibers and the tendon proper. The tendon organ lies “in-series” with this small cluster of muscle fibers, and it is therefore subjected to mechanical strain when these muscle fibers are active.

3.2.2. ENCODER MECHANISMS

We do not yet know precisely the means by which muscle force is transduced by the tendon organ receptor, but it is likely to be mediated by regional strain on nerve terminals, as they are compressed among the tendinous fascicles. It is also important to note that the tendon organ is not located within the body of the tendon itself, but within the muscle, at the muscle fiber-tendon boundary. Furthermore, tendon organs may be scattered through many muscles in regions quite distant from the tendon, although their locations are often near collagenous tissue planes.

3.2.3. TENDON ORGAN RESPONSES TO MUSCLE FORCE CHANGE

Tendon organs are excited most readily by active force increases, such as are produced by neural activation of muscle, rather than by passive muscle force increases, such as are induced by muscle stretch. Typically, under conditions of physiologic activation, tendon organ discharge increases more or less proportionately with increasing force, and the tendon organ will follow changes in force quite closely. Figure 10B shows a typical sequence of excitation of two different tendon organs, showing that each begins to discharge at some particular threshold force, and their rate increases irregularly at low forces, depending upon the recruitment of particular muscle fibers belonging to the subset of fibers attached to the tendon organ receptor. Once many muscle fibers are active, the impact of added muscle fiber activity on tendon organ discharge is diminished, and the discharge rate of the tendon organs follows the changing force quite accurately.

Although recordings of tendon organ afferents in intact conscious animal or human models are relatively rare, a number of studies have shown that

tendon organ activity in physiologically activated muscle is closely reflective of instantaneous muscle force recorded at the tendon, with relatively little sensitivity to the rate of change of muscle force. This pattern of response is summarized in Fig. 10C, which shows that rate of tendon organ discharge

increases linearly with increasing force over much of the force range, although the force-rate relation often displays an upward convexity at higher forces. This relative linearity and sensitivity of the tendon organ (TO) response is unexpected, given that the numbers of muscle fibers sampled by any

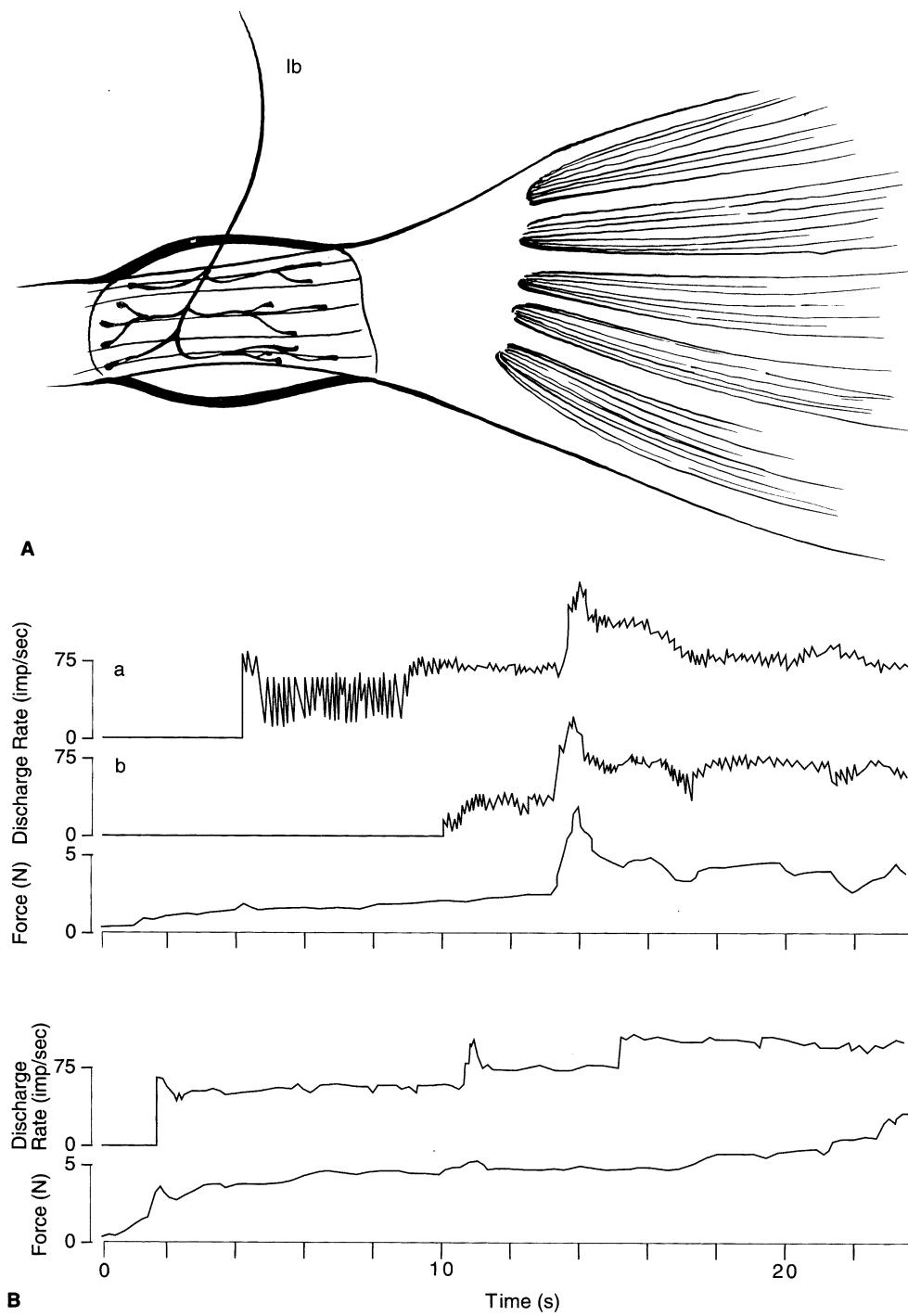


Fig. 10. (Continued)

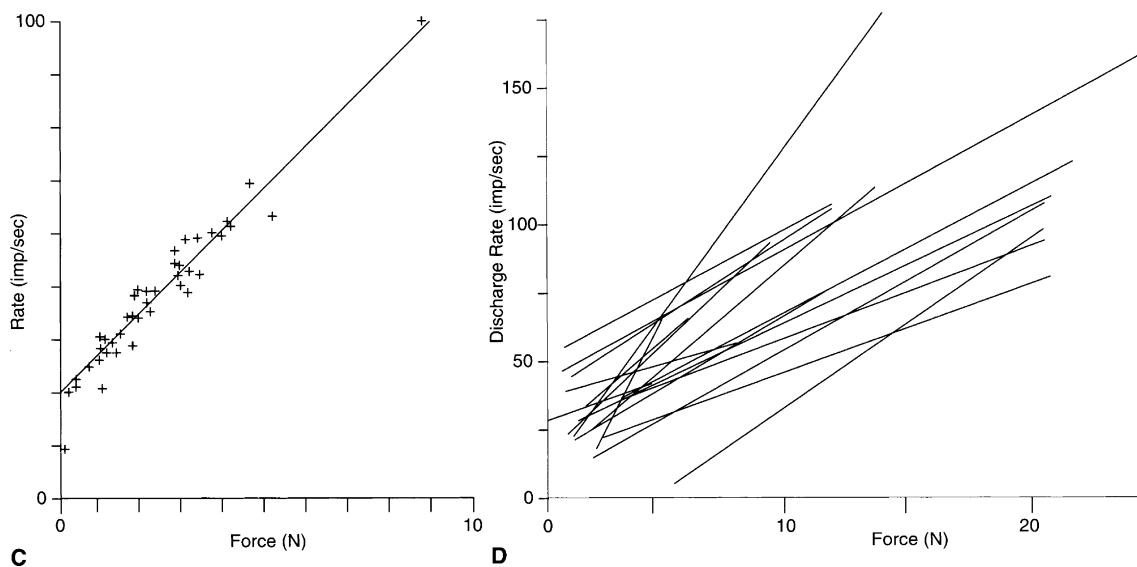


Fig. 10. (A) Expanded view of a Golgi tendon organ, showing the encapsulated receptor area around the tendon fascicles and the limited number of muscle fibers attaching to those collagen fascicles. (B) The sequence of activation of the two tendon organs was recorded from the same soleus muscle in a decerebrated cat preparation. Although the thresholds of the two receptors are somewhat different and the initial discharge rates are somewhat variable, the rates become quite smooth and follow accurately the minor fluctuations in force after a significant force level is achieved. (C) There is a straight line relation between reflexively generated static isometric force and the discharge rate. The relation is not strictly linear, because there is a non-zero intercept on the ordinate. (D) The graph summarizes the relations of a population of tendon organ afferents; the data was drawn from the soleus muscle in different preparations.

tendon organ is relatively small; typically 12 to 16 muscle fibers/tendon organ in large limb muscles of the cat (Fig. 10A). In spite of this rather limited sample, the response patterns of individual tendon organ are surprisingly close to the force variations of the whole muscle, as registered at the tendon (Fig. 10B), except at very low forces where only one or two of the muscle fibers attached to the tendon organ may have been activated.

3.3. Other Muscle Mechanoreceptors

The major muscle spindle and tendon organ afferents usually constitute less than 50% of the total sensory innervation from a typical muscle, as estimated from the total number of afferent fibers in the muscle nerve. There is also a substantial population of unmyelinated fibers in the nerve, whose receptor terminals have the appearance of free nerve endings, which are distributed throughout the muscle, including the muscle and tendon surfaces. These afferent fibers show differing conduction velocities, including a few belonging to the most rapidly conducting afferent fiber group (group I), however, in most cases these free nerve ending afferents exhibit conduction velocities of small myelinated (group III) and group IV fibers (which are largely unmyelinated). Although some of these afferent fibers arise

from specialized nerve terminals such as Pacinian corpuscles, most of them originate in free nerve endings, which have no discernible specialized terminal structure on light microscopy.

Although the nerve terminals of free nerve endings are not visibly specialized structurally on light microscopy, they do exhibit a range of functional specialization. Some terminals are responsive to nociceptive input, whereas others respond primarily to mechanical stimuli, such as pressure or tension, although with much less sensitivity than that of the encapsulated receptors (spindle and tendon organs) described earlier. Some other small afferents arise from receptors that respond to thermal and metabolic changes in the muscle, which may indicate that they have a role in cardiovascular and neuromuscular responses to exercise, as well as in neuromuscular compensation for fatigue.

4. AFFERENT PATHWAYS TO THE SPINAL CORD

4.1. Afferent Classification

As summarized in Table 1, muscle afferents display a range of diameters and conduction velocities. The most rapidly conducting fibers, which are also those

Table 1
Muscle Afferent Nerve Fiber Diameters and Conduction Velocities

Type of afferent	Mean nerve fiber diameter (μm)	Mean nerve fiber conduction velocity (m/s)	Functional roles of afferents
Group I	15	90	Primary muscle spindle endings (Ia) Golgi tendon organs (Ib)
Group II	8	48	Secondary spindle endings
Group III	4	24	Free nerve endings, myelinated
Group IV	<1	1	Free nerve endings, unmyelinated

with largest diameter (group I), typically conduct at velocities up to 100 m/s or more. In the cat, in which such fibers are most extensively studied, conduction velocities and diameters are well established and primary endings afferent conduction velocities may reach 120 m/s. Secondary spindle afferents conduct at velocities ranging from 24 to 72 m/s and small myelinated and unmyelinated fibers of skin and muscle (groups III and IV) from less than 1 m/s to 24 m/s. Conduction boundaries for the different fiber populations are not as well defined in man, and in particular, there is not a clearly defined conduction velocity boundary between primary and secondary spindle afferents.

4.2. Central Connections and Central Projections of Afferent Pathways

Sensory fibers enter the spinal cord primarily via the spinal dorsal roots, although there are known to be a small number of unmyelinated nerve fibers and even a few myelinated fibers that enter through the ventral roots. The anatomic distribution of the major afferent pathways within the spinal cord is well-known and is described in more detail in Chapter 9.

As shown in Fig. 11, large myelinated muscle afferents (groups I and II) entering through the dorsal roots tend to segregate medially as they enter the spinal cord and then travel ventrally through medial portions of the dorsal spinal gray matter before diverging to make synaptic connections with neurons in the intermediate spinal gray matter and ventral horn. Some fibers terminate by making synaptic connections on regional interneurons, whereas others continue on to make synaptic connections directly with motoneurons in the ventral horn (see later).

Small myelinated (group III) and unmyelinated (group IV) fibers make synaptic connections with neurons in dorsal gray matter, including the most superficial regions of spinal gray, called lamina I, which is largely concerned with the processing of

pain-related or *nociceptive* information (Fig. 11A). Other mechanoreceptor group III and IV afferents project to deeper regions of the dorsal gray (lamina IV, V) before they are propagated to other spinal and supraspinal sensory systems.

4.3. Spinal Circuitry

4.3.1. GENERAL FEATURES OF SPINAL INFORMATION PROCESSING

Cutaneous and muscle afferent information follows several routes in the nervous system. Large myelinated afferents enter the spinal cord via the dorsal roots and then may follow one of two distinct paths. Large myelinated afferents from skin, muscle, or joint may branch to send the fibers into dorsal column and dorsolateral column white matter, where they may travel rostrally for many centimeters. (Some of these large afferents may reenter dorsal gray matter in proximal segments where they may synapse with regional neurons, new postsynaptic fibers reentering dorsal and dorsolateral columns.) A small number of fibers may traverse dorsal white matter without relay all the way to dorsal column nuclei (gracile and cuneate). Muscle afferents in particular may circumvent gracile and cuneate relays and may make a separate relay in the brain stem (although this relay has not yet been shown to be important in the primate). The fibers from gracile, cuneate, and the brain-stem muscle afferent relay nuclei then pass to higher centers, including thalamus, cerebellum, and eventually cortex. The alternative afferent destination is directly to interneuronal and motoneuronal circuits, as described earlier.

Muscle Ia afferents make extensive monosynaptic connections with virtually all motoneurons innervating the muscle from which the spindle afferents originate (i.e., the homonymous motoneuron pool) and with many motoneurons from nearby synergists. The result is extensive divergence of afferents to many

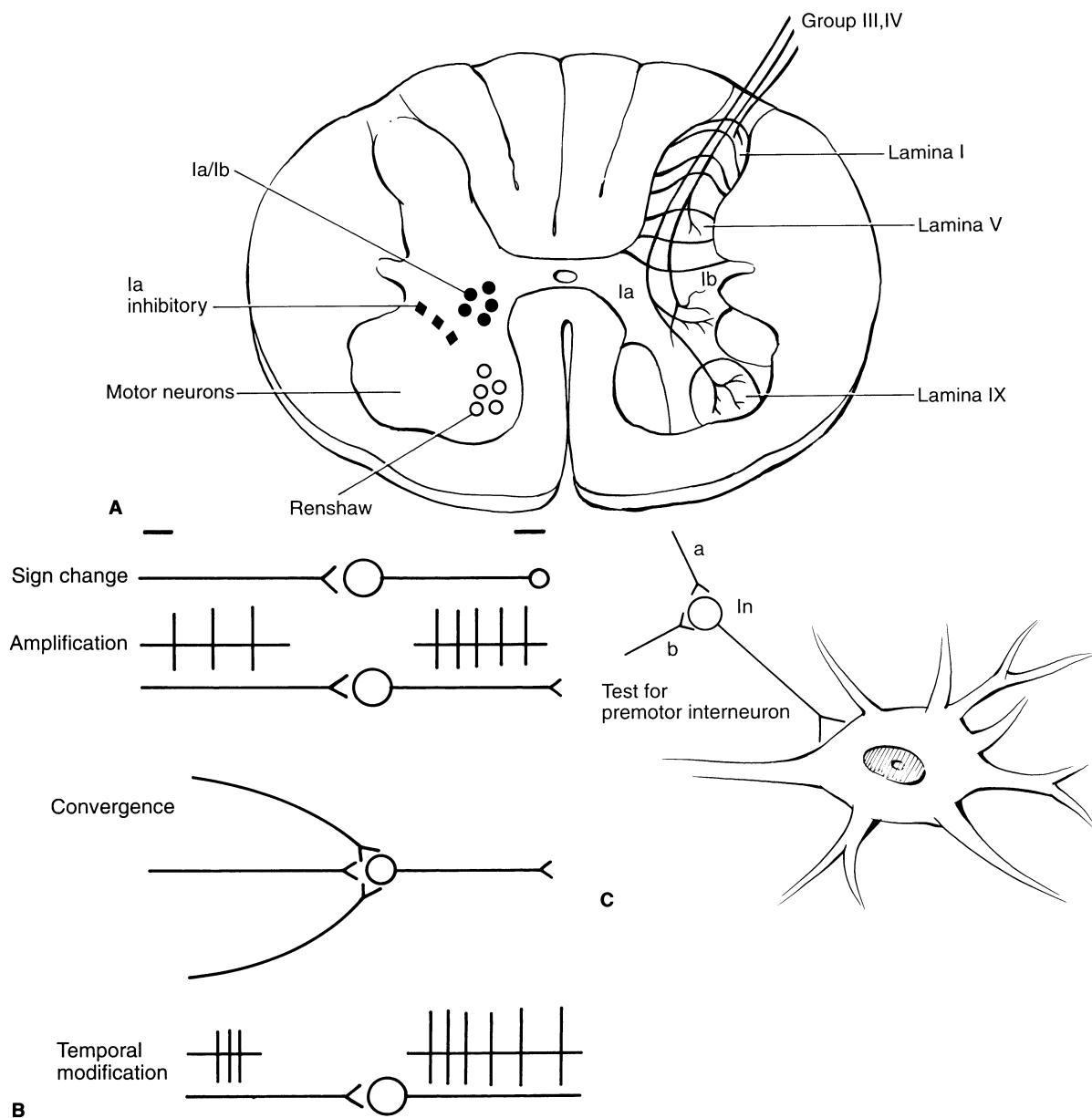


Fig. 11. (A) Interneuronal circuitry and afferent connections of the spinal cord: The spinal cord gray matter is composed of a variety of neurons that have specialized features demonstrated on light microscopy. These features have been used to classify the regions of the gray matter into the 12 laminae, with lamina I located more superficially in the dorsal gray matter. These laminae are useful for describing the preferred destination for particular classes of muscle afferent fibers. Large-diameter muscle afferents (i.e., Ia and Ib) travel medially through dorsal gray matter. Ib fibers terminate in the intermediate gray matter in laminae V through VII. Group Ia fibers may emit collaterals before proceeding into the ventral horn toward laminae IX, the location of spinal motor neurons. The cross-sectional drawing shows the major neuronal elements of the spinal cord, including spinal motor neurons, Renshaw interneurons located medial to the motor neuron core, Ia inhibitory interneurons, and Ia and Ib inhibitory interneuronal groups located dorsal or medial to the motor neuron pool. **(B)** Types of computational operations performed by spinal interneurons: The most common operation is a sign change, in which an excitatory input, such as an afferent, activates an inhibitory interneuron, which induces inhibition at its postsynaptic sites. Interneurons can amplify their input by transforming a low-frequency input train to a high-frequency output. Interneurons may also integrate spatially by means of a convergence of afferent inflow from different sources onto a particular interneuron. They may also induce changes in the temporal pattern, such as changing a transient input consisting of a few impulses into long-lasting discharge. **(C)** Key factors underlying a commonly used test for a premotor interneuron. Activation of afferent a or b individually may not be sufficient to cause the interneuron to reach the threshold, and the subsequent synapse at the neuron reveals no synaptic potential. However, if a and b are activated simultaneously, the interneuron is recruited and postsynaptic potentials are manifested in the spinal motor neuron.

motoneurons and extensive convergence of Ia afferents onto individual motoneurons.

4.3.2. SPINAL INTERNEURONAL SYSTEMS

Spinal interneurons are defined largely on anatomic grounds. Interneurons are simply neurons whose axons extend relatively short distances within the cord, usually no more than a few spinal segments. The cell bodies of these neurons are usually of small diameter, less 50 μm , and many neurons are even smaller than this. Although muscle afferents from muscle spindles (primary and secondary spindle afferents) make some direct, or *monosynaptic*, connections with spinal motoneurons in the ventral horn, almost all afferents, including those from primary and secondary endings, make their first synaptic connections with neurons in dorsal or intermediate spinal gray matter.

4.3.3. INTRINSIC PROPERTIES OF SPINAL INTERNEURONS

Spinal interneurons have a full complement of ligand- and voltage-gated channels. In most cases, these channels have not been investigated in detail. All interneurons generate action potentials via the standard mechanism, fast activating and inactivating Na channels and fast activating K channels. Multiple other types of K currents as well as Ca channels are present on interneurons, though few details are as yet available. Interneurons typically fire at higher rates than do motoneurons (whose properties are described in Section 5) but also display heterogeneous intrinsic firing patterns in comparison with motoneurons. Some interneurons appear to be capable of firing only single spikes to sustained inputs, whereas others display bursts of firing regularly much like motoneurons. The relation between these firing patterns and the diversity of voltage-gated channels is an active research area.

4.3.4. TRANSMITTER SYSTEMS

Most interneurons are inhibitory in their postsynaptic effects and release inhibitory transmitters such as glycine or GABA from their presynaptic terminals. Others are excitatory, presumably releasing glutamate, aspartate, or other excitatory amino acids from their presynaptic terminals. The effects of these various transmitters are described in more detail elsewhere in this volume.

4.3.5. INTERNEURONAL INFORMATION PROCESSING

Interneurons are an important component of spinal information processing, because they perform several important computational operations. Figure 11B

illustrates the most common types of operation performed by interneurons. The most common computational operation is a *sign change* (i.e., inhibition), in which, for example, an afferent originating from muscle or skin produces synaptic excitation at the first synaptic relay, then activates an inhibitory interneuron, producing inhibition at subsequent postsynaptic sites.

Excitatory interneurons may also act to *amplify information* received from the periphery, or they may change the *spatial distribution* of that information by virtue of the pattern of divergence of their nerve terminals. Interneurons may also change the time course and the frequency content of incoming signals, by *filtering* out high frequencies or by providing *integrator* kinds of operations. Interneurons may provide other signal processing, such as *reshaping* or changing the transmitted signal from a step to a more transient, or pulse-like, response.

Because the input from a single afferent is sometimes modest, it may be necessary to sum the input from several sources to reach the interneuron threshold. A single afferent fiber input may not elicit an interneuron response by itself but may reach neuron threshold when two or more afferent inputs are excited simultaneously. Furthermore, if these afferent sources are widely dispersed, the interneuron may serve to integrate spatial information as well.

As shown in Fig. 11C, the subliminal effect of single afferents on interneurons is useful as a diagnostic test for the existence of an interneuron in a projection pathway from afferent to motoneuron. For example, an interneuron is believed present when activation of an individual afferent pathway (such as a peripheral nerve) gives rise to *no* synaptic potential in a motoneuron, yet simultaneous activation of two afferent inputs does induce a visible synaptic potential (which could be either an excitatory postsynaptic potential [EPSP] or inhibitory postsynaptic potential [IPSP]).

Alternatively, some interneurons are specialized for spinal relay; these are called *propriospinal neurons*. Finally, others may promote *rhythm generation*, especially in the more proximal lumbar spinal segments, where they may contribute to the cyclical motoneuron excitation that takes place as part of locomotion.

In summary, the primary role of interneurons appears to be to act as summing, or integrating, elements in which convergent input from a variety of sources, sometimes including differing sensory modalities, is integrated and passed on to the next step of information processing.

4.3.6. ACTIONS OF IDENTIFIED INTERNEURONS

Muscle afferent information is relayed to interneurons in the dorsal horn or intermediate gray or to motoneurons in ventral gray matter with either monosynaptic or oligosynaptic connections in each location. The majority of the interneuronal elements of the spinal cord have not been fully identified in mammalian preparations. Only a small number of different interneuron types have been characterized, primarily because of the availability of specific and practical diagnostic electrophysiologic tests. The recent development of genetic markers for specific types of spinal neurons shows great promise for advancing our understanding in this area. New genetic techniques allowing regional- and time-specific knockout of function of identified genetic classes bring exciting new tools to the understanding of spinal circuits. As yet, the genetic markers reflect early cues for spinal neuron differentiation and development and are thus broader than known categories based on inputs and outputs. For example, two of the best studied classes of interneurons, Ia inhibitory neurons and Renshaw interneurons (Fig. 11A), initially develop together, and thus both are part of the V2 subclass of ventral interneurons. Thus knockout of V2 neurons does not identify Ia interneuron function. Such studies are only now beginning and have focused primarily on locomotion (see later). Further work identifying markers later in development will allow greater resolution (e.g., separate identification of Ia and Renshaw cells). Thus the introduction of genetic methods into the study of reflexes and control of muscle length promises to revolutionize the field. The following subsections describe the few classes of interneuron that have been clearly identified using traditional techniques of electrophysiology and anatomy (Fig. 11 and Fig. 12).

4.3.6.1. Renshaw Neurons. Identification of interneurons has been based on a variety of circumstantial electrophysiologic findings, which may either be unrelated or only obliquely related to the functional role of these interneuronal systems. For example, Renshaw interneurons, which are small interneurons located in the ventral horn medial to the spinal motor nuclei, display a unique high-frequency bursting discharge after antidromic excitation of spinal motor axons in the ventral root. These interneurons receive excitatory input from motor axon collaterals and make synapses on regional spinal motoneurons.

The transmitter released at the motoneuron axon collateral to Renshaw neurons is acetylcholine, which is to be expected, as this transmitter is also released at the other terminal branches of the motor axon at the neuromuscular junction. The cholinergic postsynaptic receptors on the Renshaw neuron are both *muscarinic* and *nicotinic* in type. Antidromic excitation of the Renshaw neuron is only partly impaired by separate administration of nicotinic or muscarinic acetylcholine antagonists but essentially eliminated by the simultaneous administration of both cholinergic blockers. For example, combinations of cholinergic nicotinic antagonists (such as mecamylamine, and dihydro β erythroidine) and muscarinic antagonists (such as atropine) produce a severe reduction of antidromically elicited Renshaw activity, confirming the diversity of postsynaptic cholinergic receptor types on the Renshaw neuron.

Axons of Renshaw neurons make synaptic connections with regional motoneurons, as well as other interneurons, including Renshaw neurons of other motor neuron pools and regional Ia inhibitory interneurons. The transmitter released by Renshaw axon terminals is believed to be glycine, a naturally occurring amino acid. There has been some suggestion that GABA may also be released at Renshaw terminals, but this has not been independently verified.

4.3.6.2. Ia Inhibitory Interneuron. Another well-described interneuron is the *Ia inhibitory interneuron*. This interneuron is identified by the findings that it receives monosynaptic excitatory input from Ia afferents in one muscle and then makes inhibitory synaptic connections to motoneurons of opposing, or antagonist, muscles. By virtue of these connections, the Ia interneuron is believed to promote *reciprocal innervation*, in which agonists and antagonists acting about a joint are prevented from being active simultaneously.

Although the functional role and utility of Ia inhibitory interneurons is relatively easy to comprehend, the identification of these interneurons is circumstantial and depends upon the fact that they are inhibited by antidromic ventral root excitation, apparently via Renshaw neurons. In other words, an interneuron activated by Ia afferents of a particular muscle but silenced by ventral root stimulation meets the necessary defining criteria.

The transmitter released at Ia interneuron axon terminals is believed to be *glycine*. Nonspecific antagonist effects are mediated by the agent strychnine, but the strychnine effects are not localized and

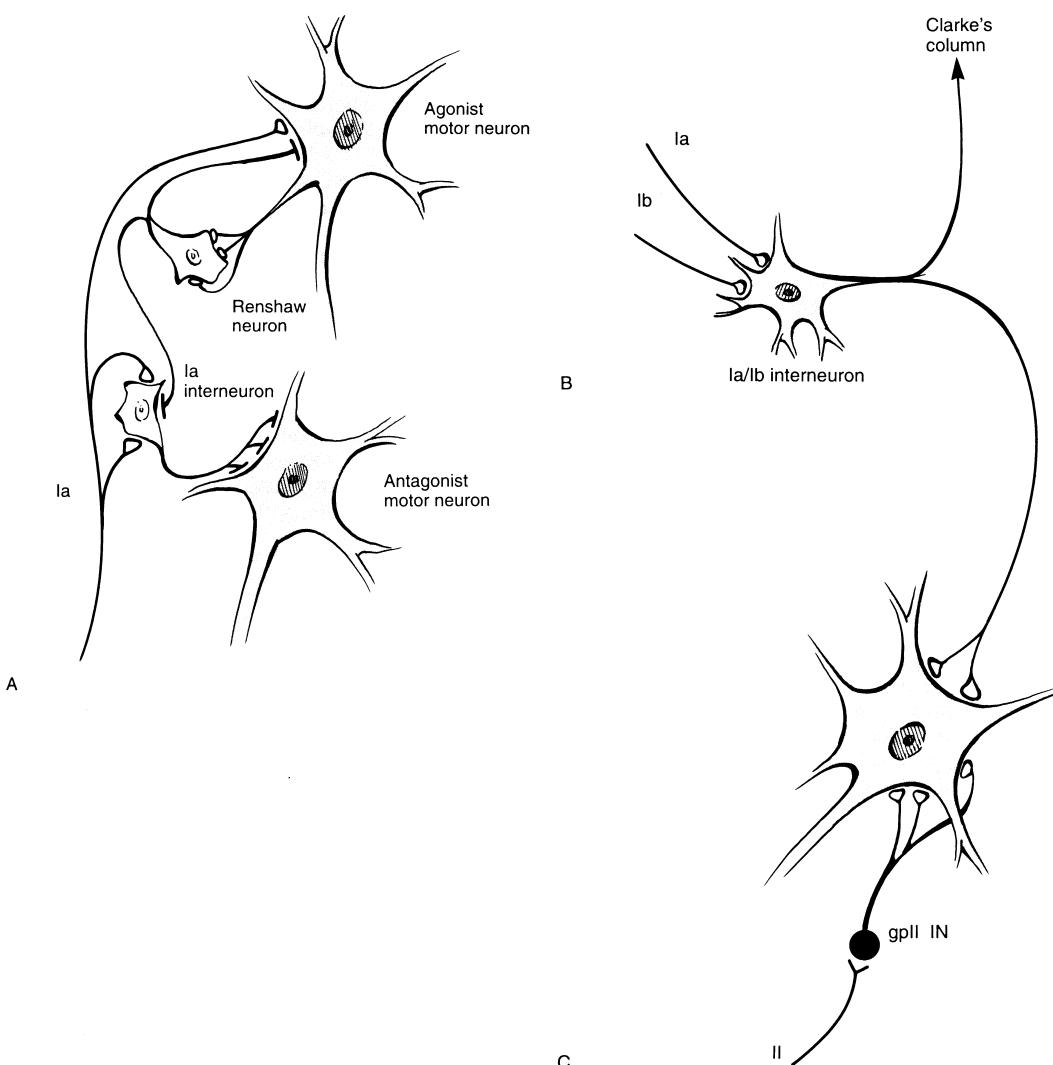


Fig. 12. Types of spinal interneurons. **(A)** This important circuit involves linkages among motor neurons and flexor and extensor muscle groups associated in interneuron pools. Flexor motor neurons produce collaterals that innervate and excite Renshaw interneurons. Renshaw axons may inhibit the originating motor neuron, the flexor, and the Ia inhibitory interneuron. The Ia inhibitory interneuron receives Ia afferent input from a flexor, which induces excitation of that flexor motor neuron. Activation of the Ia inhibitory interneuron inhibits the antagonist extensor motor neuron. Excitatory synaptic terminals are drawn as *enclosed circles*, and the inhibitory terminals are presented as *bars*. **(B)** The convergence of Ia and Ib afferents on an inhibitory interneuron inhibits the activity of a typical extensor motor neuron. The Ia and Ib interneurons also project to Clarke's column, which is the nucleus of origin of the dorsal spinocerebellar tract. **(C)** Group II afferents excite an interneuron, located in the proximal lumbar segments of the cord, which induces excitation of motor neurons in the lower lumbosacral segments.

in fact may impact many synaptic locations within the central nervous system.

4.3.6.3. Ia/Ib Interneurons. Golgi tendon organ afferents (identified as Ib afferents) are known to make synaptic relays in intermediate gray matter of the spinal cord, producing autogenetic inhibition of homonymous or synergist motoneurons. It is now apparent that these interneurons, which were

originally called *Ib*, also receive muscle afferent input from *Ia* spindle receptor afferents, making their integrative function more complex than originally perceived. Nonetheless, the major driving input is still primarily from Golgi tendon organs, which raises the possibility of a *force regulatory action* of this interneuronal system (see later).

These interneurons also project to Clark's column neurons, which lie in proximal lumbar segments and give rise to axons traveling as the dorsal spinocerebellar

tract. The existence of an inhibitory connection from muscle Ib afferents, via the Ib inhibitory interneuron to homonymous and other synergist motoneurons, raises the possibility of a closed-loop force regulator, in which force increases sensed by tendon organs give rise to inhibition of homonymous motoneurons. This force regulation has been difficult to demonstrate as a practical entity, because those animal preparations that allow study of intact reflex spinal pathways, such as the decerebrate cat model, show substantial suppression of many segmental inhibitory interneuronal systems, including the Ia/Ib inhibitory pathway.

There have been several studies performed in man that provide indirect evidence for the operation of a force regulator. These studies have used fatigue as the test probe to evaluate force feedback compensation, relying on the fact that fatigue induces a substantial short-term loss of muscle contractile force. However, although fatigue is the most common source of a loss of force-generating capacity, there are also likely to be substantial changes in afferent inflow from several types of muscle receptors, which may exaggerate the degree of responsiveness of the Ib pathway compared with the normal state. Specifically, muscle fatigue induces changes in muscle temperature, pH, and metabolic state, all of which may change the spontaneous discharge patterns and the mechanical responsiveness of group III and IV muscle afferents. Because free nerve ending afferents and Ia and Ib afferents may all converge on the same set of Ib interneurons, alterations in the baseline discharge of group III and IV afferents might well alter the responsiveness of the Ib pathway.

4.3.6.4. Group II Excitatory Interneurons. An extended series of studies has demonstrated that there exist interneurons that receive selective input from secondary spindle afferents, which make excitatory synaptic connections to lumbosacral spinal motoneurons, and which are located in proximal lumbar spinal cord segments of the cat. The functional role of this interneuronal system is not yet understood, although interneuronal structural and electrophysiologic properties and connections appear to be clearly defined. At minimum, these interneurons have the potential to spread information about muscle length from one muscle to another, but the quantitative function of this processing remains unclear.

4.3.6.5. Flexion Reflex Pathways. There is (as yet) no uniquely identified set of interneurons involved in flexion reflexes. Nonetheless, it is apparent that many

of the interneurons located in the intermediate gray matter of the spinal cord and in deeper laminae are involved in processing information from cutaneous, subcutaneous sensory endings, and from high threshold muscle afferents, especially those of smaller diameter (groups II, III, and IV). Excitation of any of these afferents often results in a coordinated pattern of flexor muscle activation. Although the range of afferent input eliciting flexion reflexes is diverse, the ultimate effect is relatively stereotyped, in that activation of many of these afferent systems induces consistent excitation of flexors and inhibition of extensor muscles primarily via the Ia inhibitory interneuronal system.

The individual neuronal elements of the flexion withdrawal reflex system are not clearly separable, and there appears to be an extensive polysynaptic chain involving multiple relay sites. The transmitters of such systems are also not clearly identified, although again the excitatory synaptic connections are likely to be mediated by excitatory amino acids (glutamate and aspartate), and it is likely that GABA plays an important role in mediating inhibition.

4.3.6.6. Presynaptic Inhibitory Interneurons.

There are also known to be interneurons located in dorsal gray matter of the cord, which terminate on presynaptic terminals of afferents and of other interneurons. These interneurons release GABA, which depolarizes presynaptic terminals and reduces the amount of transmitter released by each incoming action potential. This reduction of transmitter release is mediated by reducing the amount of calcium that enters the terminal with the arrival of each action potential.

5. MOTONEURONS AND MOTOR UNITS

5.1. Introduction

The physiology of CNS neurons began with the study of spinal motoneurons, primarily by John Eccles and colleagues in Canberra, Australia, in the 1950s and 1960s. For many years, the motoneuron was the prototype for the study of central neurons, and it still remains one of the most extensively studied neurons in the mammalian nervous system.

5.2. Definitions

As illustrated in Figure 13A, a spinal motoneuron is unusually large, with a huge dendritic tree. It is the neuron directly innervating skeletal muscle, primarily via axons traversing the ventral roots (Fig. 13B). All of the motoneurons innervating a given muscle are

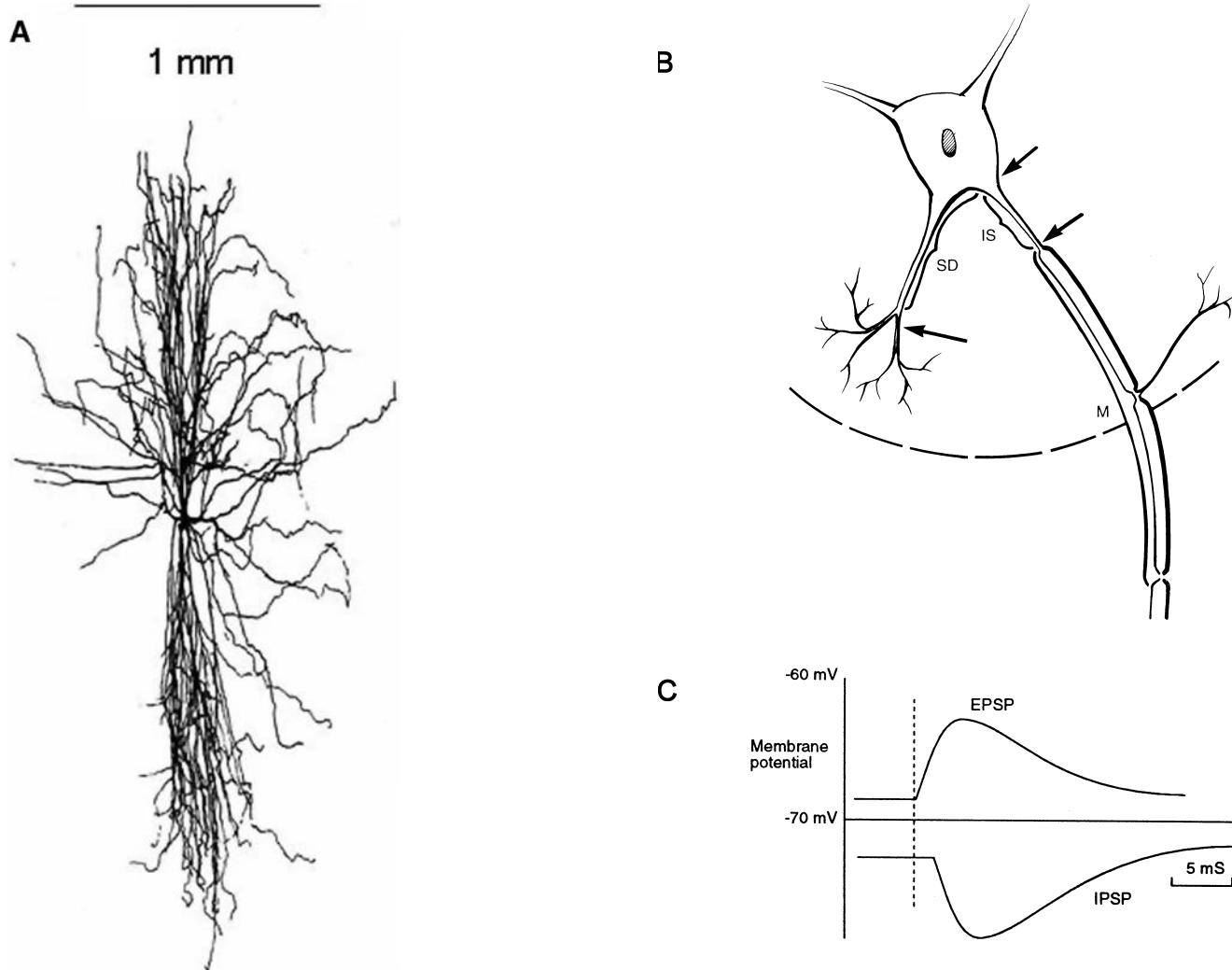


Fig. 13. (A) Motoneurons, like most neurons, have amazingly complex dendritic trees. The cell shown on the left comes from the upper cervical cord and innervates a neck muscle. Motoneurons are also unusually large. (B) Diagram of a motor neuron, showing the cell soma and dendrites (SD segment), the initial segment or axon hillock, and the first node of Ranvier. The changes in action potential formation result as a motor neuron is stimulated antidromically. At -87 mV , only a small potential, called the M potential, is visible; as the potential reaches -80 mV , a partial action potential, called the A spike, is visible, indicating invasion of the initial segment of the motor neuron. As the motor neuron potential is further depolarized to -78 mV , a full action potential is visible, with a clear inflection still evident at the transition between A and B spikes. (C) The diagram plots the time course of an excitatory postsynaptic potential (EPSP) and inhibitory postsynaptic potential (IPSP). Both have a relatively similar rise time, reaching a peak in 3 to 5 ms, and a prolonged decay. The inhibitory postsynaptic potential begins a few milliseconds after the excitatory potential because of the intervening inhibitory interneuron.

together termed the *motoneuron pool*. Each motor axon innervates a group of muscle fibers in the muscle. The motoneuron together with the innervated muscle fibers is called the *motor unit*. The group of muscle fibers innervated by one motor axon is called the *muscle unit*.

5.3. Intrinsic Properties of Motoneurons

Spinal motoneurons are of two general types. The first, characterized by a relatively large cell body (or

cell soma), innervates muscle fibers in skeletal muscle. This type of motoneuron is referred to as α , or *skeletomotor*, neurons. A group of smaller motoneurons called γ , or *fusimotor*, neurons that innervates muscle spindles (see earlier discussion of muscle spindle efferent innervation) is also distinguishable. α and γ motoneurons are interspersed throughout the ventrolateral portion of spinal gray matter. An additional class of neurons called β , or *skeletofusimotor*, motoneurons (described earlier) is also present in

many vertebrate systems, but these neurons have no features distinguishing them structurally or physiologically from α motoneurons, at least so far as we know. β motoneurons are characterized solely by the fact that they jointly innervate skeletal muscle and intrafusal fibers of muscle spindles in the same muscle.

Although the soma of spinal motoneurons may be quite large, approaching 100 μm or more, the dendritic arbor is even larger and may extend for several millimeters radially from the cell soma out into white matter and far up into dorsal gray.

Spinal motoneurons are characterized by a range of specific ligand- or voltage-gated conductances.

5.3.1. LIGAND-GATED CONDUCTANCES

Synaptic input to motoneurons functions like that to all neurons: neurotransmitters (the ligand) are released by the presynaptic bouton and bind to the postsynaptic receptor. This binding then opens the postsynaptic receptor to allow ions to flow. EPSPs are produced by excitatory neurotransmitters such as glutamate and IPSPs by inhibitory ones, with glycine being the main one in the spinal cord but GABA also being important. As shown in Fig. 13C, the synaptic potential begins quite quickly after the arrival of the action potential in the presynaptic nerve terminal. (This small delay is important because it indicates that the effects are not mediated by direct electrical transmission.) After an interval of about less than 1 ms, there is a rapidly depolarizing voltage change, which may reach a peak in 3 to 5 ms and then decay gradually over the ensuing 15 or 20 ms.

From a variety of biophysical studies of motoneurons, it is now evident that the time course of the synaptic potential is dependent jointly upon the magnitude and time course of synaptic current injection (which may last only 100 to 300 μs) and on the electrical properties of the cell membrane (the latter is referred to as the resistance-capacitance [RC] properties of the membrane). The excitatory synaptic potential follows its particular time course, primarily because of charge storage and distribution on the membrane “capacitor.” This membrane charge is then slowly dissipated over the subsequent 10 to 20 ms, with a time course that depends on the electrical properties of the membrane and on the physical location of the synaptic terminals on the cell surface.

Specifically, the effective current flow seen at the axon hillock, the preferred site of action potential initiation of the motoneuron, is strongly influenced

by membrane properties, and by the cell geometry, especially the pattern of dendritic branching. In particular, excitatory synaptic potentials that originate on distal dendrites are greatly attenuated and filtered by the electrical properties of the cell. It is now very clear that the dendrites of motoneurons are not passive conductors of these synaptic currents but contain multiple voltage-sensitive conductances that amplify synaptic current (see the following section).

When some inhibitory inputs are activated, such as those from Ia inhibitory interneurons or from Renshaw cells, the IPSPs will begin 1 to 2 ms later, because of the time needed to activate one or more intervening interneurons, but the synaptic potentials have a broadly comparable time course, although with hyperpolarizing voltage changes. A typical sequence and time course of these synaptic potentials is diagrammed in Fig. 13.

5.3.2. VOLTAGE-GATED CONDUCTANCES

In addition to the ligand-gated conductances described above, there are a number of voltage-gated conductances. As in all neurons, these conductances are differentially distributed over the initial segment, soma, and dendrites of the motoneuron. The rapid activating Na and K conductances that generate the action potential are highly concentrated in the first node of Ranvier and on the regional membrane close to the axon (the axon hillock), but they are relatively less concentrated over the cell soma and the dendrites. This means that when current is injected into a cell, the first point at which action potential generation is initiated is at the axon hillock and the first node, and this is then followed by the retrograde invasion of the action potential into the soma and dendrites (as well as the standard orthodromic invasion of the motor axon). This discontinuity in activation sequence is visible during standard intracellular recordings as a minor inflection on the rising edge of the voltage recording of the action potential.

High-threshold calcium (Ca) channels allow for a significant rise in intracellular Ca levels during the action potential, activating a Ca-sensitive potassium (K) channel that generates a medium duration after-hyperpolarization (AHP) after each spike. This medium AHP (50 to 200 ms) is much longer than the fast AHP (2 to 4 ms) generated by the K channel that repolarizes the action potential. As noted above, motoneuron dendrites now appear to contain both inward and outward voltage-sensitive channels. Two main types of currents have been demonstrated to

have a dendritic origin. Both currents are persistent, in that they remain open in response to continued input. A small percentage (~1% to 3%) of the Na channels that mediate the action potential fail to inactivate and thus generate a persistent Na current. This current forms about half of the total persistent inward current (PIC) in motoneurons. Recent results suggest that a significant portion of the NaPIC arises in dendritic regions, though the exact location is unknown. The second component of the PIC is mediated by an unusual type of Ca channel, an L-type channel (genetic subclass, Cav 1.3; genetic subclasses for other channels on motoneurons remain uncertain at this point). Cav 1.3 channels activate at very hyperpolarized levels, often below the voltage threshold for the spike. It is likely that the majority of the Cav 1.3 channels are dendritic, and indeed the majority of the current of the PIC in motoneurons has been shown to have a dendritic origin. The dendritic PIC plays a major role in amplifying and prolonging synaptic input (see Section 6.3.1). PICs and several other voltage-sensitive channels are subject to regulation by neuromodulatory inputs, especially those that release the monoamines serotonin and norepinephrine.

5.3.3. THE FREQUENCY-CURRENT FUNCTION

The motoneuron is an exceedingly complex electrical structure, with a diverse set of ligand- and voltage-gated channels distributed on both its soma and extensive dendritic tree. Its basic electrical behavior is, however, reasonably simple: as current injected via a microelectrode at the soma is linearly increased, a distinct threshold for repetitive firing of action potentials is reached (Fig. 14). Further increases generate approximately linear increases in firing rate, forming what is called the primary range. At higher levels, a secondary range is reached, which is probably due to activation of the PIC. PIC activation occurs at much lower levels when synaptic instead of injected current is employed, because much of the PIC is located in dendritic regions right where the synaptic input enters the cell. Because the PIC becomes larger and its voltage threshold lowered with appropriate neuromodulatory input, the secondary range in normal movement conditions likely begins at or below threshold for the frequency-current function (Fig. 14, left-hand function). The frequency-current function is the core behavior of the motoneuron and is a major determinant of its firing pattern during reflex and volitional inputs (see Section 5.5).

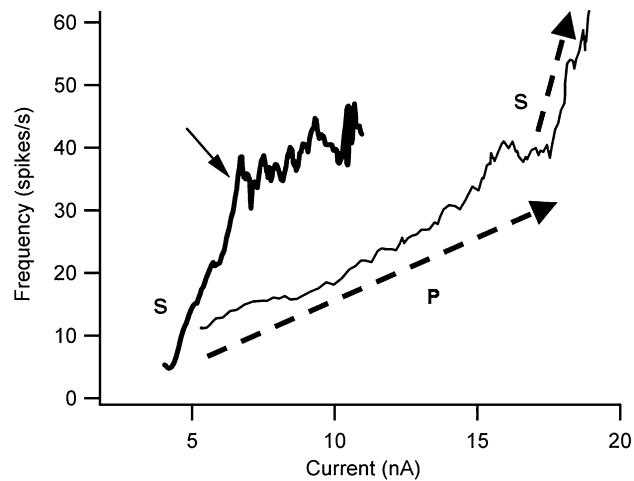


Fig. 14. The frequency-current function of motoneurons obtained by injecting current via a microelectrode inserted in the cell, with minimal neuromodulatory input (*thin trace, right*). It has a sharp threshold, and then an approximately linear increase in firing rate known as the primary range. A steeper slope is sometimes reached at higher levels, known as the secondary range (S). Activation via synaptic input and a background of neuromodulation by descending axons releasing the monoamines serotonin or norepinephrine bring the secondary range down to the threshold level (*thick trace, left*). This occurs because the secondary range is primarily generated by persistent inward currents in dendritic regions that are facilitated by the monoamines. At the arrow, activation of the PIC is complete and slope becomes much more shallow. This region is sometimes referred to as the tertiary or preferred firing range.

5.4. The Motor Unit: Electrical-Mechanical Transduction

Whenever a motoneuron fires an action potential, whether by natural synaptic input or an electrical stimulus applied to its axons, the muscle fibers innervated by this motor axon are excited and generate a twitch. The mechanical features of this twitch are determined by the number and mechanical properties of the innervated muscle fibers. There has been extensive study of these motor unit properties and of the relation between the mechanical behavior of the muscle fibers and the electrophysiologic properties of the innervating motoneuron. A number of these findings are summarized in Fig. 15 (see Section 5.5.2).

Motor units vary in the size and time course of the twitch elicited by a single stimulus and in the resistance to fatigue manifested during repetitive activation. The magnitude of the motor unit twitch varies greatly between different mammalian muscles, and for different motor units in the same muscle,

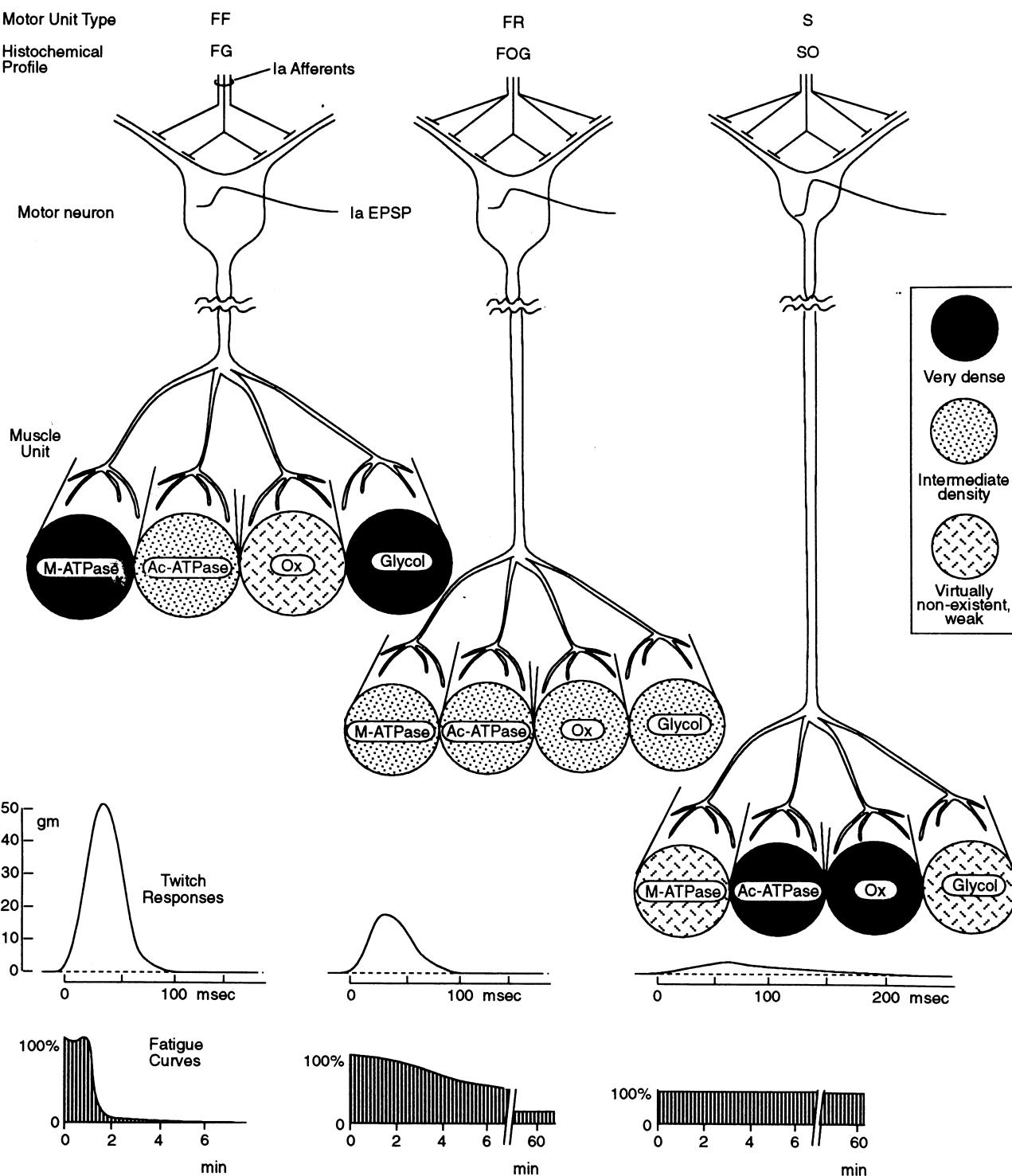


Fig. 15. Relations between motor neurons and the muscle fibers they innervate. Three different-sized motor neurons innervate muscle fibers of varying diameters (*upper section*). The twitch is induced by transient motor neuron and has the lowest peak force and slowest rise time for the smallest motor neuron. Repetitive activation of the large neuron (*left*) results in a force tetanus that declines very rapidly, decaying almost completely within 2 min, whereas the smaller neuron (*right*) generates a smaller tetanus, which does not decline over many minutes; the intermediate unit shows a partial decline in tetanic force. These differing degrees of fatigue sensitivity are used in conjunction with differences in twitch contraction time to classify motor units (*see upper table and text*). Metabolic correlates of the physiologic differences are also shown (*central portion*). S-type units show dense concentrations of oxidative (Ox) and mitochondrial (Ac-ATPase) enzymes. FF units stain strongly for glycolytic enzymes. These staining differences support an alternative histochemical classification scheme (*top*); fast glycolytic (FG), fast oxidative glycolytic (FOG), and slow oxidative (SO). The largest neurons receive the most extensive Ia afferent input, but with low terminal density; whereas the smallest motor neurons receive the highest density of Ia afferent input.

however, much of our current knowledge is drawn from the hindlimb muscles of the cat, especially the medial gastrocnemius (MG). These studies have shown that many muscles, such as the MG, are quite heterogeneous, possessing units with a spectrum of mechanical and biochemical properties.

5.4.1. TWITCH SIZE

When the twitch responses of different units in a given muscle are compared, the twitch tension, or force amplitude, varies from several grams weight to only a few milligrams. The reasons for these differences are complex, but there are a number of factors contributing to the differences in twitch tension.

First, large motor units could have more muscle fibers innervated by a motor axon. Expressed differently, the number of muscle fibers innervated by a single motor axon, which is called the *innervation ratio*, could be larger in those units generating larger tensions. In the case of the MG muscle, the innervation ratio for large twitch units is indeed greater than that for small units. In MG, for example, the innervation ratio may vary from 250 to 700 muscle fibers per axon, a 3-fold range, but the differences in force may vary by 50- or even 100-fold.

A second factor contributing to the generation of muscle force is the *cross-sectional diameter of the muscle fiber*. The cross-sectional diameter is important because it reflects the number of myofibrils that can contribute to force generation, as these myofibrils are arranged in parallel and can add to net fiber force independently. The average cross-sectional area of individual muscle fibers in large twitch motor units is substantially greater than that of small twitch units.

The third and final factor is the specific force-generating capacity of each fiber type, or the *specific tension*. It is possible that fibers belonging to different types of motor units are capable of generating different forces, even when the effects of innervation ratio and of cross-sectional area are eliminated. In other words, the *force/unit area*, which is the measure of specific tension, is potentially greater in fast-twitch than in slow-twitch fibers. Attempts to calculate the specific tension of different muscle fibers have been made, and they show at most a threefold difference between fast- and slow-twitch units, however, the accuracy of such estimates is open to question, and the contributions of specific tension are likely to be modest.

5.4.2. TWITCH TIME COURSE

Different motor units vary greatly in the speed of their contraction, which is measured from the onset of the twitch transient to the peak of the twitch. This speed of contraction turns out to be an important marker of unit specialization and an excellent correlate and predictor of the fatigability of the unit during repetitive activation.

For the case of the cat medial gastrocnemius, units are broadly divisible into *fast* and *slow twitch*, with a boundary value of 55 ms. Units with twitch contraction times longer than 55 ms are called *slow-twitch* units, or S-type. These units are able to generate only small twitches and modest levels of tetanic force during repetitive activation. The *fast-twitch* units, or F-type units, have contraction speeds less than 55 ms and are usually able to generate much larger twitches and correspondingly greater tetanic forces. However, they are not able to sustain this tetanic force without change during prolonged repetitive activation (see Section 5.4.3).

The physiologic basis of the differences in twitch contraction times is not fully established, however, the use of an array of histochemical techniques has revealed a systematic difference in the staining of muscle fibers for a particular myosin ATPase. As described previously, this ATPase is located on the myosin head and may regulate the speed of cross-bridge recycling during muscle fiber excitation. Current evidence does not support the view that this ATPase is entirely responsible for regulating the speed of twitch contraction, but it may be implicated as one component of the regulatory process.

5.4.3. FATIGABILITY OF MOTOR UNITS

When a motor unit is subjected to repetitive activation at high frequency (such as 30 pulses per second for 300-ms bursts), the unit generates a sustained tetanus, whose force magnitude may be several fold greater than that of the twitch. This force response to prolonged repetitive activation has proved to be a helpful classification tool. To illustrate, when an S-type motor unit is activated repetitively, the force response reaches a sustained tetanus and remains at the same level for prolonged periods of time, often up to several hours.

When these motor unit fibers are examined with histochemical stains, these S-type fibers contain many mitochondria and stain heavily for mitochondrial enzymes (such as succinic dehydrogenase and mitochondrial ATPases). These S fibers also contain substantial concentrations of myoglobin and are surrounded by a dense capillary network. There is usually relatively little intracellular

glycogen stored. The enzymatic profile indicates that these fibers are specialized for oxidative phosphorylation and depend on transported blood glucose or free fatty acids to generate the necessary ATP. Because oxidative phosphorylation is an efficient means to generate ATP production, muscle contraction can continue without decrement for prolonged periods of time, provided that blood flow is sufficient to deliver the needed metabolic substrates. In humans, it is difficult if not impossible to identify the fibers belonging to a single motor unit, and all fibers belonging to type S motor units are considered to be type I (see Section 1.5.1).

In contrast, fast-twitch units show a broad range of mechanical behaviors during repetitive activation and a correspondingly broad range of metabolic/enzymatic features on histochemical analysis. During repetitive excitation, some motor units fail to sustain the tetanic force for even 2 min, falling to less than 25% of the initial level. They are classified as *fast-twitch, fatigable* (FF) units. Other fast-twitch units are able to sustain their tetanic force more readily, falling to between 25% and 75% of the initial tetanic force at the 2-min mark. These are called *fast-twitch, fatigue-resistant* (FR) units.

Histochemical analyses of these fast-twitch units show an array of histochemical profiles. The most fatigable units (FF) show high concentrations of myosin ATPase, few mitochondria, little or no myoglobin, a meager capillary network, and substantial glycogen stores. These units appear to rely on glycolysis to generate the necessary ATP for sustaining contraction and are therefore called FG units in current metabolic classification schemes. In humans, the muscle fibers for FG motor units are equivalent to the type IIx discussed previously (see Section 1.5.1).

The units showing *intermediate degrees of fatigability* reveal some residual oxidative machinery on histochemical analysis, including mitochondria and associated mitochondrial enzymatic staining. Because the histochemical profile is mixed, these units are classified as FOG in metabolically based schemes (type IIa in humans; see Section 1.5.1).

It appears that the degree of fatigue, estimated from the loss of force, is related to the capacity to sustain high levels of ATP production. In FF (FG, IIx) fibers, ATP synthesis declines when glycogen is lost and muscle contraction declines, whereas in FR (FOG, IIa) fibers the residual oxidative contributions delays fatigue onset and thus they behave more like S motor units (type I fibers).

5.5. Motor Units as a Functional System

5.5.1. INTRODUCTION

There are two broadly different ways in which activation of the motoneuron pool can increase muscle force. The first mode is *recruitment* of motoneurons, which is simply the transition from a passive state, in which the motoneuron is quiescent, to an excited state, in which the motoneuron is emitting action potentials. The second mode of force regulation is achieved by *increasing the rate of discharge of individual motoneurons*. This is called *rate modulation*. In effect, the first option progressively activates more and more motoneurons (and muscle fibers), progressively filling in all the elements in the pool until the pool is completely recruited. The second option, *rate modulation*, alters the individual force output of single motor units by virtue of their capacity to produce a partially fused tetanus, in which the mean force output becomes progressively greater with increasing rate of motoneuron discharge. Recruitment and rate modulation are closely tied to the threshold and slopes of the motoneuron frequency-current functions (see Section 5.3.3).

In life, these two regulatory mechanisms are closely interwoven, with recruitment being the dominant source of force increase at low levels of motoneuron pool excitation and rate modulation generating a progressively greater and greater impact on muscle force output as more and more motoneurons are activated.

5.5.2. RECRUITMENT AND THE SIZE PRINCIPLE

When a motoneuron belonging to a particular motoneuron pool is subjected to increasing excitatory synaptic input, such as from muscle spindle Ia afferent fibers, the resulting progression of excitation and recruitment in different motoneurons is very orderly and virtually stereotypic, obeying a principle called the *size principle*. This principle, enunciated first by Henneman and colleagues in 1965, states that motoneurons are recruited in a defined rank order, in which small-sized motoneurons are recruited first, and these are followed progressively by larger and larger motoneurons. Conversely, if motoneurons in a pool are already active, the application of an increasing inhibitory input, such as from antagonist muscle Ia afferents, causes a derecruitment of motoneurons, in which the largest motoneurons are the first to drop out. With increasing inhibition, derecruitment continues progressively with the smallest motoneurons being the last to be silenced.

5.5.2.1. What Is Meant by Size of Motoneuron? The original description by Henneman of the relation between recruitment rank order and size was derived from studies using ventral root recordings of motor axons, in which the size of the extracellular action potential recorded from the ventral root was used as an indication of the size of the motor axon, and by inference as an indication of the size of the associated motoneuron. A typical pattern of activation is illustrated in Fig. 14C, which shows that increasing muscle extension gives rise to recruitment of ventral root action potentials of progressively greater and greater amplitude. This correlation between action potential size and motoneuron somatic size is broadly applicable to the bulk of motoneuron axons recorded in the ventral root, but it may not hold in fine detail. This is partly because unrelated technical factors may influence the size of the recorded action potential but mostly because motoneuron size itself may not be the primary factor governing motoneuron recruitment order (see later).

For example, when motoneuron recruitment order is plotted against tetanic tension of the associated motor unit, the relation becomes much clearer and is more linear than is the relation between recruitment rank order and axon action potential size, and recruitment “reversals,” in which the larger motoneuron appears to be the first activated, are much less common. Although tetanic motor unit force has no obvious causal relation to neuronal factors governing recruitment, it presumably provides a more sensitive marker of net motor unit properties than is available by relying simply on action potential size.

5.5.2.2. Physiologic Mechanisms of the Size Principle.

The physiologic factors responsible for the orderly recruitment of motoneurons by size are not yet fully understood, in spite of intensive and continuing investigation. Originally, Henneman proposed that motoneuron size itself could be the governing factor. The idea was that for a given synaptic current, applied to all motoneurons in the pool, the magnitude of the resulting excitatory synaptic potentials in motoneurons would be determined by the effective electrical *input resistance* of the motoneuron, which is inversely related to the surface area of the cell. All other things being equal, a small neuron would present a higher input resistance than would a large neuron because of the reduced membrane area available to transmit the current, and the resulting excitatory potentials in smaller motoneurons would be expected to be larger. Because neuron voltage

threshold is essentially constant in different motoneurons, the differences in EPSP size would then dictate the order of recruitment. An equivalent way of looking at recruitment is to consider the synaptic current required to reach the threshold for the frequency-current function. Because resistance varies with size, the current thresholds of small neurons are much smaller than those of large neurons. The range of differences in the threshold for the frequency-current function is about 10-fold. A substantial portion of this is indeed due to cell size, though differences in resistance per unit area of membrane probably also contribute. The changes in motoneuron electrical properties mediated by the monoamines are also correlated with cell size, because the dendritic Ca current that generates amplification of synaptic input and bistable behavior has a substantially lower voltage threshold in smaller motor neurons.

Many synaptic inputs are distributed among motoneurons in a nonuniform fashion, with some inputs systems tending to generate larger synaptic currents in larger motoneurons (e.g., descending input from the rubrospinal nucleus and some cutaneous inputs). These inputs do not appear to be capable of reversing the normal order of recruitment. Therefore, it appears that the large differences in the intrinsic electrical properties of motoneurons maintain the size principle no matter what the organization of synaptic input.

It is important to realize that the intrinsic electrical properties of motoneurons correlate strongly with the properties of the muscle units that they innervate: threshold currents correlate directly with muscle unit force and contraction speed and inversely with fatigability. This point is emphasized in Fig. 15. Consequently, low-force, highly fatigue resistant units are invariably recruited first. Put another way, the size principle of recruitment states that first S, then FR and then FF units are recruited. This sequence maximizes the fatigue resistance at any force level. It also maximizes precision (smallest units first) and energy efficiency (S units are most efficient because of their slow contraction speeds). This consistent pattern of recruitment thus matches the mechanical demands of the wide diversity of movement patterns.

5.5.3. RATE MODULATION

There is some tendency for the firing rate of motoneurons at their recruitment threshold to vary systematically with recruitment rank order. This is very clear with injected current during intracellular recording but may be obscured by synaptic noise during normal motor behavior in humans. In

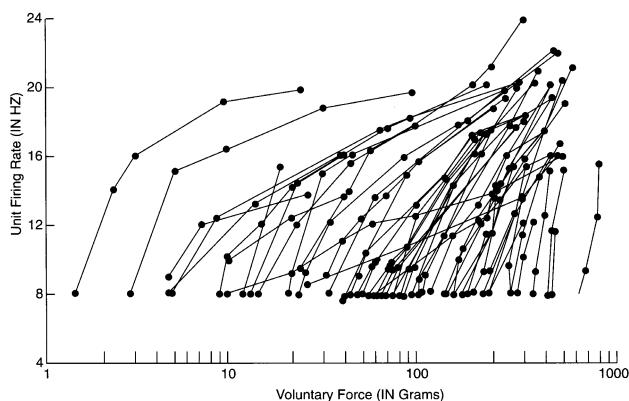


Fig. 16. Regulation of muscle force: Recruitment of motor neurons is manifested as activation of the elements in the motor neuron pool. Each connected set of dots in this figure shows the firing rate of an individual motoneuron. In humans, where these data comes from, firing rates of all units are similar at recruitment threshold (*initial dot* for each motoneuron). Rate modulation is available at all levels of recruitment. The discharge rate of the motor neuron strongly influences the net force output of the motor unit. At very low discharge rates, individual twitches generated by motor neuron excitation do not summate significantly, but at rates exceeding 6 or 8 pulses per second, a partially fused tetanus develops. Under normal conditions, motor units operate in a response region in which their tension output is partially fused.

Fig. 16, for example, no systematic differences in initial rate are evident. Perhaps the most striking feature of motoneuron rate modulation in humans is the phenomenon of *rate limiting*, in which low-threshold units undergo a dramatic reduction in firing rate as higher-threshold units are recruited (Fig. 16). This is likely due to the activation of the dendritic PIC, which imparts an initial step slope followed by a much shallower one to the motoneuron frequency-current function (see Section 5.3.3; Fig. 14). As argued below (see Section 7.4), such PICs are likely a natural component of normal motor behavior due to neuromodulatory input. Rate limiting aids in energy efficiency, as the slow-twitch fibers innervated by early recruited motoneurons are already driven effectively by the low rates achieved before rate limiting commences.

5.5.4. INPUT-OUTPUT RELATIONS FOR A MOTOR POOL AND THE MUSCLE IT INNERVATES

The size principle of recruitment, which is due primarily to the differences in electrical properties of the motoneurons themselves, means that the pool of motoneurons innervating a single muscle act together as a single functional system. The input-output function of this system cannot be directly measured due to

a variety of technical limitations, but computer simulations based closely on motor unit properties show that its basic form is sigmoidal (see Fig. 19 and Section 7.4). The initial upward curvature is due to recruitment of units with progressively larger forces, and the final smooth approach to maximum reflects the similar trend in the force-frequency functions of individual motor units (see Section 2.1.1).

6. NEUROMODULATION OF SPINAL CIRCUITS

6.1. Introduction

The number of neurotransmitters shown to be active in the CNS has grown tremendously in the past two decades. The vast majority of neurotransmitters act via a diversity of receptor subtypes, which can be broadly separated into two main categories. Ionotropic receptors are the classic ones that mediate EPSPs and IPSPs. These receptors work by opening and allowing flow of ions. In contrast, neuromodulatory receptors (also known as metabotropic) activate G protein-coupled receptors, which in turn activate intracellular signaling cascades. Intracellular signaling pathways are known to be complex and involve multiple molecules and feedback loops. This section will primarily focus on four neurotransmitters that have received considerable study and have been shown to have strong effects on spinal circuits.

6.2. Main Neurotransmitters with Neuromodulatory Actions

The monoamines serotonin (5HT) and norepinephrine (NE) are released by axons originating in the brain stem and project to all parts of the spinal cord. Both act via a number of different receptor subtypes. Best studied are the 5HT₂ and NE alpha₁ receptors, which have very potent excitatory actions on motoneurons, and 5HT₁ and NE alpha₂ receptors, which have inhibitory actions on a variety of interneurons and also mediate presynaptic inhibition of high-threshold afferent input.

The classic ionotropic excitatory neurotransmitter glutamate also acts on neuromodulatory receptors, which are known as metabotropic glutamate receptors (mGluRs). These exist in multiple forms, both excitatory and inhibitory. At present, the best understood mGluRs act to facilitate spinal interneurons in the dorsal horn that probably process medium- to high-threshold afferent input. Finally, the classic inhibitory neurotransmitter GABA acts via GABA_A receptors to generate IPSPs and GABA_B receptors to

mediate neuromodulatory actions. Activation of GABA_A receptors reduces input from group I muscle afferents, and the GABA_A agonist baclofen is a potent antispastic agent in humans. It appears to mediate its actions primarily by presynaptic inhibition, though postsynaptic effects on voltage-sensitive channels may also be involved.

6.3. Descending Monoaminergic Systems

6.3.1. FACILITATION OF MOTONEURONS

On motoneurons, both serotonin and norepinephrine have several facilitatory actions on voltage-sensitive channels, mediated by 5HT₂ and NE alpha₁ receptors. These actions include marked enhancement of both Na and Ca PICs (see Section 5.3.2), hyperpolarization of spike voltage threshold, depolarization of the resting membrane potential by closing K channels, and reduction of the AHP after the spike. The effects on PICs are especially strong, resulting in a transformation in the way the

motoneuron integrates synaptic input. In low-threshold type S cells, input is both amplified and prolonged (both effects are shown in Fig. 17). In higher-threshold FR and FF motoneurons, primarily amplification occurs. The mechanism of these effects is as follows. Most of the PIC is generated in the extensive dendritic regions. As a result, a brief input to the cell can evoke a sustained output. If the sodium (Na) conductances that generate the action potential are blocked, the effect of the PIC is revealed as a sustained depolarization known as a plateau potential. In normal conditions without blockade of Na channels, the brief input evokes a long-lasting train of action potentials, giving self-sustained firing without input. Self-sustained firing is confined mainly to type S motoneurons where the resulting tonic firing likely forms a fundamental component of posture. Equally if not more important is the amplification of synaptic input while it is still being applied. Recent studies show that the dendritic PIC that this voltage-sensitive dendritic conductance can amplify excitatory input

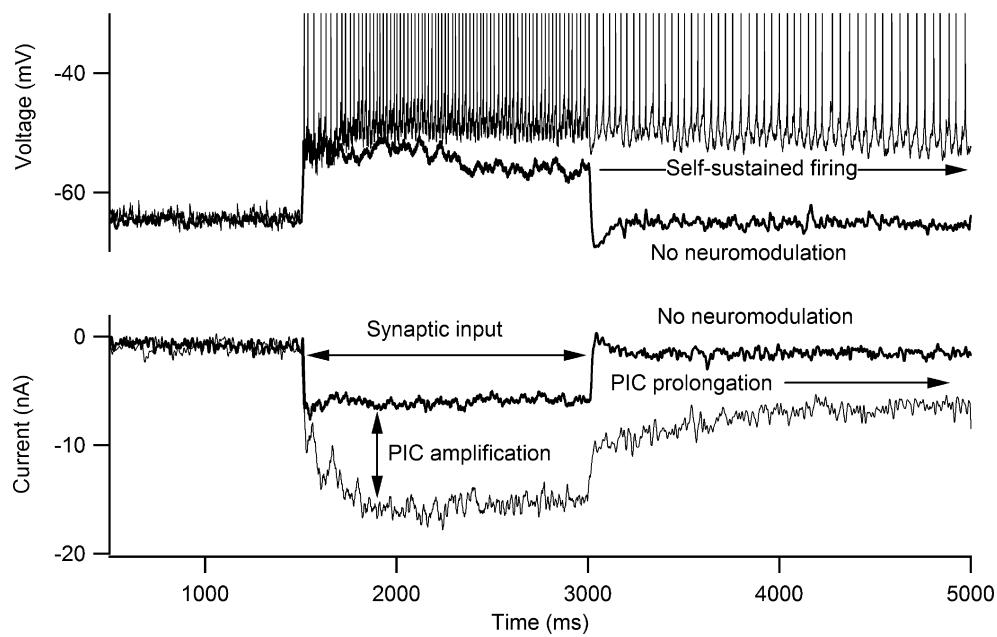


Fig. 17. Effects of neuromodulatory input from the brain stem on a motoneuron receiving a steady Ia synaptic input from vibration of its muscle tendon. When no descending neuromodulation is present, the synaptic current (*bottom panel*) has a sharp onset and offset. With moderate neuromodulation due to serotonin or norepinephrine, the synaptic current is markedly increased (amplified) and prolonged by the persistent inward current (PIC). These currents cause the depolarizations illustrated in the *upper panel*: with no neuromodulation, the synaptic potential (essentially a series of EPSPs summed together to produce an approximately steady depolarization, *lower trace*) does not quite reach threshold for firing. The PIC amplification produces much stronger depolarization, causing the cell to fire strongly. The PIC prolongation keeps the cell firing after the synaptic input ceases (self-sustained firing). This behavior is typical of that of a low threshold, type S motoneuron. FR and FF motoneurons exhibit the same degree of amplification but much less prolongation.

by a factor of 2- to 10-fold, depending on the level of monoaminergic drive. As the brain stem cells that release the monoamines are tonically active in the waking state, it is highly likely that amplification and self-sustained firing play a major role in defining motor neuron output during normal motor behavior (see Section 7.4).

6.3.2. MIXED EFFECTS ON SPINAL INTERNEURONS

Facilitory effects of serotonin and norepinephrine on spinal interneurons appear to be weaker than on motoneurons and confined to interneurons in the intermediate and ventral portion of the cord that process information from group I and II muscle afferents. In the dorsal horn, inhibition predominates, involving 5HT1 and NEalpha2 receptors. These inhibitory actions likely involve both presynaptic inhibition and postsynaptic reductions in intrinsic excitability via facilitation of K channels. The group II interneuron system spans both effects, receiving a mixture of excitation and inhibition. As a result, descending monoaminergic systems tend to facilitate low-threshold muscle afferent input and inhibit high-threshold input (see Section 7.4).

6.4. Local Neuromodulatory Systems

There exist many other neuromodulatory systems in the spinal cord, just as in other parts of the CNS. To a large degree, the functional role of these systems has not been elucidated. Metabotropic glutamate receptors may be particularly important in regulating the action of interneurons in the intermediate and dorsal portions of the cord, but the source of input to these receptors is unclear. Cholinergic systems are likely to also have strong actions, and indeed motoneurons have large cholinergic synaptic terminals linked to control of K conductances. Much further research is needed into the interaction between descending monoaminergic systems, which have been clearly demonstrated to have powerful effects, and local neuromodulatory systems.

7. SPINAL REGULATION OF MOVEMENT

7.1. Introduction

To this point, this chapter has described the spinal elements that are implicated in control of muscle force, including the neurons of the spinal cord, the muscle receptors, and the muscle itself. The sections that follow will address the functional contribution of these various elements to the control of movement.

Broadly speaking, the spinal cord is engaged in three aspects of movement regulation.

- Information transmission: The spinal cord *relays afferent information* to higher centers in spinal cord, brain stem, and beyond, and *transmits efferent commands* from higher centers to the motor nuclei of spinal cord.
- Reflex action: Spinal cord neurons and their connections form the substrates for a variety of *sensory-motor reflexes*.
- Pattern generators: Spinal and brain-stem interneurons form the basis for *oscillatory neuronal discharge*, which underlies rhythmical behaviors such as *locomotion, respiration, and mastication*.

Each of these functions is subject to control by neuromodulatory actions mediated by the serotonergic and noradrenergic axons descending from the brain stem.

7.2. Definition of Reflexes

A reflex is defined as a stereotypic motor response to a particular sensory input. Reflexes vary broadly in the complexity of their motor response and in the number and diversity of neural elements that are used. Reflexes may be relatively simple, involving sensory afferents such as Ia afferents from one muscle, inducing activation of motoneurons innervating the same muscle. Such reflexes, which include the *tendon jerk* and the *tonic stretch reflex*, are often referred to as *autogenetic*, or *homonymous in type*. Reflexes elicited by muscle afferents of one muscle acting on motoneurons of a neighboring muscle are often described as *heteronomous* in type. When such reflexes result in coordinated responses between two or more muscles with similar mechanical actions, the muscles are said to be acting as *synergists*.

At a somewhat higher level of reflex organization, there is a “reciprocal” pattern of activation, in which inhibition is exerted by Ia afferents of one muscle on motoneurons of the antagonist muscle via the *Ia inhibitory interneuron* (see earlier description of this interneuron).

At the next level, there is a more complex array of reflexes in which the response to a sensory input may be relatively complex and even repetitive, or rhythmical in character. These reflexes include such responses as the *flexion withdrawal reflex* in which a noxious or sometimes even non-noxious stimulus to skin or other deep tissues evokes a broad-scale coordinated withdrawal of a limb by systematic activation of flexor muscles at several joints.

Beyond the flexion withdrawal reflex, there is an array of complex goal-directed reflexes such as *wipe and scratch reflexes*, in which local excitation of skin surface gives rise to a coordinated and often repetitive motion by the animal to remove the irritant focus from the skin. In mammalian quadrupeds, this is usually called the *scratch reflex*, and in amphibians it may be referred to as the *wipe reflex*. The fact that the action attempting to eradicate the irritant focus is repetitive and rhythmical in character suggests that the response may also involve an *oscillator* or *pattern generator*, which gives rise to rhythmical excitation of spinal circuits.

7.2.1. STRETCH REFLEX: SPRING-LIKE PROPERTIES OF THE STRETCH REFLEX

Since the work of Sherrington, it has been well-known that stretch of muscle in a physiologically active animal, such as an unanesthetized decerebrate preparation, gives rise to a substantial increase in motor output, which is reflected as an increase in muscle force. (This increase in force is induced by orderly recruitment and rate modulation of motoneurons in a pattern characterized in earlier sections of this chapter.) This increase in motor output results in a systematic increase in muscle force, in which the force rises smoothly and approximately in proportion to the degree of muscle extension. Conversely, when the muscle is allowed to shorten, the reduction in force is essentially proportional to the reduction in muscle length.

These features of the stretch reflex response can be characterized as being “spring-like” in character, in that a change of length is accompanied by a proportional change of force, the defining feature of a simple spring (see earlier description of spring-like properties of muscle). Furthermore, when the muscle stretch is maintained, the increase in force is also maintained, verifying the spring-like property, as a spring continues to resist when extended. However, when muscle is stretched at a constant velocity, it also shows a force overshoot at the end of the stretch, suggesting that there is some degree of dynamic, or velocity, sensitivity.

A detailed comparison of the forces generated over a broad range of velocity indicates that the effect of increasing velocity is relatively modest. Specifically, a 100-fold increase in stretch velocity induces less than 2-fold change in muscle force. When the force increment induced by stretch, measured at a constant length increment, is plotted against stretch velocity, the relation is seen to be nonlinear and is well described by a power function relation, in which force increases with velocity raised to the power of

0.2 (i.e., $F = Kv^{0.2}$). It appears then that muscle is a relatively weak viscous element, in that the increase in force is much less than proportional to the increase in velocity. It is of interest, however, that the force generated increases relatively steeply at very low velocities, so that muscle would emulate a frictional device, resisting motion most powerfully at the onset of motion, where velocities are small.

7.2.2. COMPARISON OF REFLEX AND AREFLEXIVE BEHAVIOR OF MUSCLE

To review briefly (see Section 2.1.1), when active muscle is removed from reflex control, such as by dorsal root section, muscle displays an asymmetric response to stretch, in that stiffness is modest during stretch yet is quite large in shortening. When reflex mechanisms are intact, these asymmetrical mechanical properties of muscle are fully compensated, preventing muscle yield from being manifested.

As shown in Fig. 18, if the mechanical (or areflexive) response to stretch and release is superimposed upon the reflex response (matched for the same initial force), it is evident the early phase of the force response to

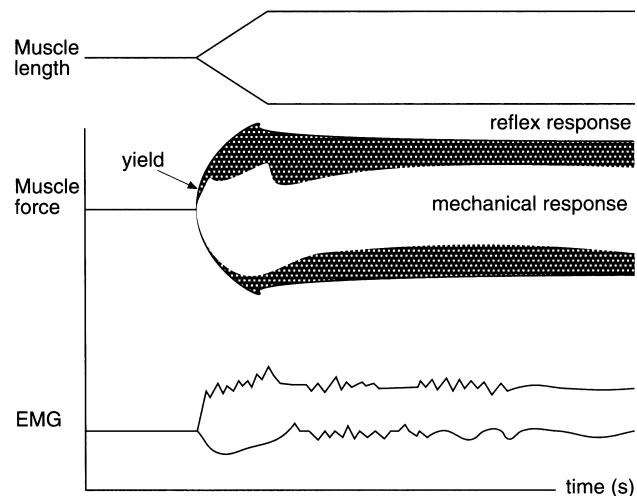


Fig. 18. Comparison of mechanical and reflex responses of reflexively active soleus muscle of the cat. Muscle is exposed to symmetric stretch and release of constant velocity. In the central part of the figure, the mechanical response is replicated, showing the short-range stiffness, yield, and asymmetric force characteristics. The overall reflex response (*top*) is much more spring-like, because stretch and release produce a smooth and progressive change in the force, which is essentially symmetric. Force changes are sustained even during the plateau phase of the length change. The electromyographic (EMG) responses are also asymmetric, reflecting the different reflex actions during stretch and release. During stretch, there is a modest EMG reduction that is relatively smaller in magnitude, although with a sustained component during the hold phase.

muscle stretch in both cases is virtually identical, indicating that the response to stretch is initially governed by the intrinsic mechanical properties of active muscle. However, at the point that yielding should have taken place, the reflex response continues smoothly without discontinuity, indicating that effective compensatory mechanisms must have been operating.

In contrast, the response to muscle shortening is much more similar for reflex and mechanical (i.e., areflexive) responses, indicating that there is less requirement for neurally mediated compensation during the shortening phase of motion. These differences in the mechanical behavior are also mirrored in the electromyographic responses, which show substantial EMG increases during stretch and relatively modest EMG reductions during shortening.

The finding that the more complex mechanical properties of muscle, such as the muscle yield, are obscured in the presence of reflex action indicates that the *reflexes serve to linearize and to smooth the mechanical behavior of muscle*. It is also clear that in the absence of reflex action, the onset of muscle yield occurs very early in relation to stretch onset, so that muscle mechanical properties would normally be expected to change abruptly, approximately within 20 to 50 ms of stretch onset. This rapid change is likely to impose severe time constraints on compensatory responses, which may take an additional 25 to 50 ms to elicit an appropriate mechanical response. A second issue is that the mechanical response to muscle is quite asymmetric, requiring substantial reflex compensatory responses in stretch, but relatively modest responses in shortening. We need to be able to explain this substantial asymmetry of reflex action.

These dual *constraints of timing and asymmetry* indicate that straightforward feedback control mechanisms are unlikely to be responsible for compensating both muscle yield and asymmetric muscle mechanical behavior, because the time constraints are too severe to allow errors to be detected, transmitted to the cord, and the corrective neural command relayed and implemented in the muscle. The debate about the nature and consequences of reflex action is still ongoing, however, we now believe that much of the compensatory response for muscle yield is built into the response characteristics of the muscle spindle receptors.

This is an unexpected solution to the problem of controlling muscle force, as muscle spindle receptors are usually designated as primary length sensors rather than as sensors regulating muscle force. Furthermore, in most published experiments, muscle length is controlled by the stretcher, so that muscle

spindle responses would be expected to be essentially invariant and independent of muscle force levels. Nonetheless, this receptor is the only candidate with a short onset time for activation, and it also has the requisite pattern of asymmetric response to stretch and release. It appears then that the neural mechanisms mediating stretch reflex action act predictively, at least initially, in that the muscle spindle receptor issues a response that is appropriate for correcting impending changes in muscle properties, such as muscle yielding and asymmetric muscle stiffness.

Although tendon organ responses could contribute to and promote the improvement of muscle mechanical properties, the speed of tendon organ-mediated feedback is too slow to prevent the manifestation of rapid-onset mechanical changes, such as muscle yielding. Furthermore, appropriate compensatory muscle mechanical responses occur even when Ib interneuron responses are very modest or even absent.

7.2.3. ASSESSMENT OF STIFFNESS REGULATION

The dual and competing actions of muscle length feedback (which would promote increased stiffness of muscle) and force feedback (which would induce reduced muscle stiffness) are simultaneously present and active under many conditions, suggesting that regulation of stiffness to some predetermined level could be a possible function of stretch reflex mechanisms. This view was advanced by Nichols and Houk (1976), who showed that the stiffness of muscle was more constant in the presence of reflex action, although the actual magnitude was not held closely to any specific value.

We now believe that the evidence in support of a primary role of the stretch reflex as a stiffness regulator is limited, because muscle stiffness does not remain constant or even approximately constant under most operating conditions. Nonetheless, it is clear that the presence of force and length sensors, together with their reflex connections, gives rise to a much more spring-like behavior of muscle, and it is this characteristic of muscle that is likely to be important in the control of movement.

For example, the presence of predictive reflex compensation means that the sharp extension of ankle extensors, such as the soleus extension, which takes place during the stance phase of locomotion, would induce a smooth force increase and enhance effective damping of the contact point.

7.2.4. FLEXION REFLEXES: COORDINATED MULTIMUSCLE, MULTIJOINT RESPONSES

Flexion reflexes are highly sophisticated movements, with a specific movement goal, withdrawal

from a painful stimulus. Although the circuitry underlying these responses is poorly understood, the movement patterns are well described. An entire limb is involved, requiring coordination of multiple muscles and joints. Moreover, these movements vary markedly depending on the source of the stimulus. For example a painful stimulus to the top of the foot evokes a movement down and away; to the bottom of the foot, up and away. The flexion reflex for limbs supporting the body evoke crossed extension to the opposite leg, to preserve balance. Thus this well-known “reflex” actually consists of a coordinated movement pattern that can take place entirely within the spinal cord.

7.3. Central Pattern Generators

Virtually all nervous systems contain groups of neurons that have the capacity to generate rhythmical bursting behavior, even when isolated from other neuronal systems. These neurons may show spontaneous bursting, or they may need a “gating” signal, such as is provided by monoaminergic agents like norepinephrine, but the pattern of discharge is not dependent on any incoming afferent or descending signals, hence the term *pattern generator*.

On the basis of extensive studies from acute and chronic spinally transected preparations (including cat and turtle), it is evident that such neuronal clusters can induce repetitive discharge sufficient to drive locomotion. In spinal cords removed from rodents and sustained in an artificial environment, stable locomotor patterns can be evoked from interneuronal circuits by appropriate tonic pharmacologic drive (a combination of serotonin and the glutamate agonist NMDA work best). Thus, locomotion-like patterns of motoneuronal discharge are not dependent on either descending, ascending, or peripheral afferent inputs. These results support the view that locomotion emerges from the activities of a discrete oscillator, located primarily within the interneurons of the spinal cord. Normally, however, the spinal pattern generator appears to be activated by descending inputs from locomotion control regions in the mesencephalon and other areas of the brain stem.

The discharge of the spinal pattern generators is subject to peripheral modulation in that it can be modified in important ways by appropriate segmental afferent inflow. Specifically, cutaneous stimulation of a foot in a quadruped during swing phase of locomotion gives rise to avoidance behavior in which the leg is caused to circumvent the obstruction, whereas when the leg is still weight bearing, similar

cutaneous stimulation is ineffectual, indicating that there is a substantial phase-dependent modulation of the effects of sensory input.

7.4. Neuromodulatory Systems and Gain of Spinal Circuits

There exists a great diversity of motor tasks and thus the capacity to adjust the excitability or gain of spinal circuits seems necessary. For example, high force or explosive movements presumably require high gain in motoneurons, whereas fine coordination may well benefit from lower gain. Descending monoaminergic systems potentially provide such control. The raphe nucleus in the brain stem, the source of serotonergic input to the cord, increases its output as motor output increases (i.e., firing rate of these neurons increases as speed of locomotion increases). The locus coeruleus (and subcaeruleus nucleus) are linked to arousal. Both systems are quiescent in sleep. Jacobs and colleagues have hypothesized that serotonergic input facilitates motor output but inhibits sensory input. Given the effects of monoaminergic input on motoneurons and interneurons discussed above (see Section 6), this overall concept is undoubtedly true. The central pattern generator for locomotion also is sensitive to monoaminergic input, though it remains unclear if this is gain control or a permissive role. In any case, facilitory effects on this pattern generator are consistent with enhancement of motor output. For sensory input, suppression of high-threshold afferents mediating the flexion reflex would prevent the large contact forces generated in locomotion from evoking inappropriate withdrawal responses. It was emphasized above, however (Section 6.3.2) that group I and II muscle afferent input is an exception to this sensory inhibition. This exception may allow the stretch reflex, reciprocal inhibition, and force feedback to remain reasonably well scaled to motoneuron excitability, maintaining overall limb stability for both low and high motor outputs.

The potency of effects of the descending monoaminergic system is very strong and can be illustrated by three examples. (1) Flexion reflexes are evoked by only the most noxious stimuli in normal conditions but become sensitive to mild stimuli in acute spinal injury where monoaminergic input is lost. (2) For motoneurons, it is very likely that a moderate level of monoaminergic input is essential for normal motor function. The self-sustained firing in type S motor units likely plays a fundamental role in maintaining muscle tone during posture. Having motoneurons generate steady firing on their own simplifies the

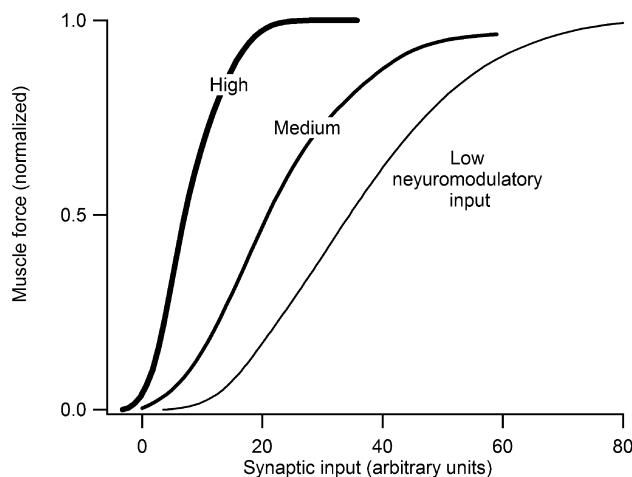


Fig. 19. Input-output functions of the set of motor units that form an individual motor pool in the cord and muscle in the periphery. The input (x-axis) represents the average synaptic input to all motoneurons in the pool and the output the summed forces of all motor units active at each input level. On the right, the low slope function represents a state of low neuromodulatory drive, as occurs during sleep or in deeply anesthetized animals. Increasing levels of descending monoaminergic drive from the brain stem progressively increase the excitability of motoneurons via facilitating persistent inward currents and other effects noted in Section 6.3.1. As a result, overall system gain increases markedly, potentially allowing the gain to vary with different motor tasks.

task for descending systems, which only need to modulate firing as needed for corrective responses. In addition, control of motoneuron excitability provides gain control via facilitating the persistent inward current as well as by the lowering of recruitment threshold by effects on the resting potential and spike voltage threshold (see Section 6.3.1). Figure 19 shows the input-output function for a whole motor pool and muscle (see Section 5.5.4) with minimal, moderate, and high monoaminergic drive to the cord. In the minimal state, excitability is so low that realistic computer simulations estimate that full activation of all synaptic input, both descending and reflex, would only produce 40% of maximum force.

This likely corresponds with the state in acute spinal cord injury where low motoneuron excitability likely contributes significantly to spinal shock. Normal movements would not be possible in this state. Fortunately, system gain is nicely proportional to monoaminergic input control of motoneuron excitability (which occurs by all the effects discussed in Section 6.3.1). The descending neuromodulatory systems thus allows system gain to be fine tuned to match different motor tasks, conferring a high degree of flexibility on spinal motor output.

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Robert D. Grubbs

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1. NEUROTRANSMITTERS, NEUROHORMONES, AND NEUROMODULATORS

Neurotransmitters, neurohormones, and neuromodulators are the chemical messengers that allow one cell to communicate with another cell or with itself. The concept of chemical messenger systems comes from studies of the peripheral nervous system, which began in the late 1800 s and focused mainly on the somatic (e.g., neuromuscular junction) and autonomic (e.g., sympathetic and parasympathetic) nervous systems. These areas provided an

accessible, discrete, and less complex model of interactions between neurons and target cells. Much of our current understanding of brain function derives from studies that began in earnest in the middle 1800 s devoted to grinding up specific organs in an effort to extract their "vital essence."

2. NEUROTRANSMITTERS ARE SMALL ORGANIC MOLECULES THAT CARRY A CHEMICAL MESSAGE FROM A NEURONAL AXON OR DENDRITE TO ANOTHER CELL OR NERVE

Even in ancient times, remarkably accurate concepts suggested that the large nerves of the body, which could be easily visualized during dissection,

carried a substance or substances that coordinated and activated the body. The Greeks and Romans called this substance *psychic pneuma*, a product of vital and animal pneuma from the lungs and heart. Early Hindus suggested that the spinal cord and the sympathetic-chain ganglia were channels, which they called *chakra*. These chakra carried a substance, called *pram*, the flow of which could be augmented by the practice of yoga. As early as 3000 BC, the Chinese taught about the flow of *chi*, an energy that flowed through the body in channels, which could be helped by the medical practice of acupuncture and by an exercise called *tai chi chuan*.

We now know that neurotransmitters are small molecules that are synthesized and stored in secretory granules or vesicles in the axons of nerves. These transmitters can be grouped chemically into families, including the biogenic amines (e.g., the catecholamines: norepinephrine and dopamine; and the indolamines: serotonin and histamine), acetylcholine, amino acids (e.g., γ -aminobutyric acid [GABA], glutamate, glycine), and small proteins (peptides, such as substance P, vasopressin, and oxytocin [OT]) (1). The peptide transmitters are generally synthesized in the cell body and carried to the axon terminal by axoplasmic transport, and the nonpeptide transmitters are generally synthesized in the axon terminal. By combining with synaptic receptors, these transmitters either inhibit or stimulate a biological response in the target cell.

The process known as *neurotransmission* refers to the passage of biochemical information by means of a chemical messenger from a nerve across a specific junction to another cell. This should not be confused with *nerve conduction*, which is the passage of an electrochemical current down a nerve axon. Table 1. lists some proposed criteria for classifying a substance as a neurotransmitter.

Table 1

Steps to Identify a Substance as a Neurotransmitter

1. Anatomic: The substance must be present in appropriate amounts in the presynaptic process.
2. Biochemical: The enzymes that synthesize the substance must be present and active in the terminal, and enzymes that degrade it must be present in the synapse.
3. Physiologic: Stimulation of the presynaptic axon should cause the release of the substance, and application of the specific substance should mimic stimulation of the nerve.
4. Pharmacologic: Drugs that affect specific enzymatic or receptor-mediated effects of the proposed transmitter substance should alter nerve stimulation through changes in synthesis, storage, release, uptake, or stimulation or blockade of the receptor.

3. NEUROHORMONES ARE CHEMICAL MESSENGERS THAT ARE SECRETED BY THE BRAIN INTO THE CIRCULATORY SYSTEM AND ALTER CELLULAR FUNCTION AT A DISTANCE

The word *hormone* was originally coined by E.H. Starling in 1905 in reference to a “chemical messenger which, speeding from cell to cell . . . coordinates the activities and growth of different parts of the body.” Thus, a neurotransmitter such as epinephrine, which is synthesized and released from the adrenal medulla, can also be a neurohormone. In the brain, certain hypothalamic neurons make small proteins, the *peptide neurohormones*. These are secreted from axons directly into a portal vasculature system, in which they are carried to the anterior pituitary to influence endocrine function. The early Greeks and the Romans ascribed a similar function to this part of the brain. Galen and later Vesalius suggested that the hypothalamus produced *pituita* (Latin for “phlegm”), which was distilled from the ventricular system and secreted through the hypothalamus into the pituitary, and then to the nose.

Just as a nonpeptide neurotransmitter can sometimes function as a neurohormone, peptide neurohormones appear to function as neurotransmitters in some cells. Many of the neurohormone-producing cells have axons that project into other areas of the brain, the brain stem, and the spinal cord to influence other somatic and behavioral functions. A full discussion of this subject is found in Chapter 9.

4. NEUROMODULATORS ARE TRANSMITTERS OR NEUROPEPTIDES THAT ALTER THE ENDOGENOUS ACTIVITY OF THE TARGET CELL

Excitable cells are unique because they have a specific complement of ion channels that dictate how these cells behave. The spontaneous behavior of cells is often referred to as *endogenous activity*. This activity is best observed when the cell or nerve is removed from its normal environment and studied *in vitro*. One of the best examples is the rhythmic activity of the heart, which is modulated by the autonomic nervous system. When removed from a frog, the heart continues to beat if it is incubated in an isotonic buffer containing calcium. The rate of contraction of the heart depends on the rhythmic activation of ion pumps that maintain a gradient of sodium and potassium across a relatively leaky membrane.

Sympathetic nerves alter the strength and rate of contraction of the heart through cardiac β receptors that modulate the production of intracellular messengers and the activity of membrane ion channels. Some neurons in the brain also have ionic pumps that rhythmically maintain an endogenous activity, or “firing pattern.” Thus, neuromodulation allows a neuron to adapt its endogenous activity to changes that occur in its environment.

Although investigators initially assumed that each neuron used only one transmitter, more recent studies have shown that some neurons produce and release more than one kind of signaling molecule. These studies have shown that the main transmitter and a co-transmitter or *neuromodulator* can be released from the same axon terminal at different frequencies of nerve activity (*vide infra*). As neuromodulators, co-transmitters serve a feedback role by combining with presynaptic autoreceptors and thereby augment or inhibit further nerve activity. In some nerves, such as the peripheral sympathetic nerves, a substance can have both transmitter and modulator functions. For example, norepinephrine can activate a postsynaptic receptor and influence target tissue function (e.g., heart rate) and stimulate a presynaptic autoreceptor (e.g., α_2) to inhibit further norepinephrine release.

An experimental technique known as *immunocytochemical labeling* can be used to visualize a transmitter or the specific enzymes needed to make a neurotransmitter within a nerve cell or axon. By using primary antibodies that are specific to the transmitter or enzyme and fluorescent secondary antibodies that bind to the primary antibodies, the transmitter and co-transmitter can be visualized within the same axon or nerve-cell body. In many cases, a typical small neurotransmitter and a neuropeptide coexist in nerves as the pair of co-transmitters. Either of the two substances may serve the neuromodulator function. For example, although norepinephrine functions as the transmitter and neuropeptide Y (NPY) as the modulator in sympathetic nerve endings, their roles may be reversed in specific neurons within the brain. A list of transmitters with documented co-transmitters is given in Table 2.

The classic small neurotransmitters are stored in synaptic vesicles (30 nm in diameter), which are manufactured and packaged within the axon terminal itself. The larger peptide co-transmitters are stored in large synaptic vesicles that are synthesized and assembled in the neuron-cell body (e.g., soma) and reach the terminal by axonal transport, a process that can take hours or days.

Table 2
Coexistence of Neurotransmitters and Modulators

Neurotransmitter	Co-transmitter
Dopamine	CCK, enkephalin
Norepinephrine	Neuropeptide Y, neurotensin, enkephalin
Epinephrine	Neuropeptide Y, enkephalin
Serotonin	CCK, enkaphalin, substance P
Acetylcholine	VIP, enkaphalin, enkephalin, CGRP
GABA	Enkephalin, neuropeptide Y, CCK
Glutamate	Substance P
Glycine	Neurotensin

CCK, cholecystokinin; CGRP, calcitonin gene-related peptide; GABA, γ -aminobutyric acid; VIP, vasoactive intestinal peptide.

The small transmitter vesicles are concentrated in an active zone of the terminal, associated with an electron-dense area of presynaptic membrane that contains many calcium channels. Low-frequency nerve stimulation causes a localized increase in calcium in the active zone that stimulates the release of the small transmitter by initiating the fusion of the anchored vesicle with the membrane. The transient

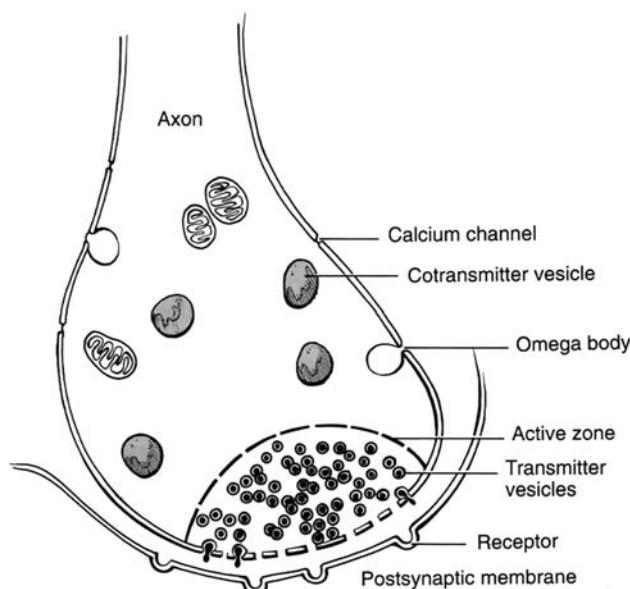


Fig. 1. The diagram of an axon terminal shows the active zone with many transmitter vesicles and calcium ion (Ca^{2+}) channels. Co-transmitters are localized in larger granules in the periphery of the terminal, where there are fewer Ca^{2+} channels. To achieve the necessary Ca^{2+} concentrations for co-transmitter release, a higher frequency of nerve stimulation is necessary.

increase in intracellular calcium is rapidly reversed by calcium binding to neuronal proteins and extrusion by being pumped out of the axon. In this way, calcium levels reach a threshold value only in the active zone during low-frequency stimulation. In contrast, the larger vesicles for the peptide co-transmitters are found in other parts of the axon terminal, distant from the active zone. Higher-frequency nerve stimulation is required to produce increased calcium concentrations in these areas of the terminal and induce co-transmitter release. Therefore, low-frequency nerve stimulation can specifically release the small transmitter in the active zone, whereas higher frequency stimulation is needed to affect co-transmitter release. These interactions are shown in Fig. 1.

5. THE RESPONSE TO TRANSMITTERS CAN BE EITHER FAST OR SLOW

Synaptic transmission can be rapid or slow, depending on the nature of the stimulus and the type of receptor involved. For example, acetylcholine acting at the nicotinic receptor and certain amino acids (glutamate and GABA) can produce a rapid alteration in postsynaptic function that occurs in milliseconds. The molecular mechanism for this rapid response is mediated through the opening or closing of ligand-gated ionic channels (2). These acetylcholine, GABA, and glutamate receptors are composed of five protein subunits that form a channel in the membrane for sodium or calcium (see Chapter 5). Activation of these receptors induces a rapid change in the ionic movement across the membrane. Whether this action persists for milliseconds, seconds, or minutes is a function of the intensity of the stimulus, the specific transmitter, and the type of receptor mediating the postsynaptic response.

Other transmitter-receptor interactions induce slower cellular responses. Acetylcholine acting on muscarinic receptors and the catecholamines produce slow changes in the target cell that can take several seconds to minutes to manifest. These changes may include phosphorylation of proteins, activation of enzymes and second messengers, or even activation of genetic transcription and production of new proteins. The receptors that mediate these responses are single-subunit, 7 transmembrane (7-TM) domain proteins that activate a G protein to initiate a biochemical response within the cell. Thus, acetylcholine, catecholamines, and other transmitters acting on G protein-coupled receptors (GPCRs)—including

neuropeptides and amino acids—can produce effects that extend over a period of hours or days.

The site of synthesis, the need for axonal transport of transmitters, and the location of enzymes needed for their metabolism are important regulatory factors for synaptic transmission. Axonal transport occurs at a rate of 1 to 400 mm/day. The peptide transmitters, which are synthesized in the cell body and require transport to the axon terminal before release, could be easily depleted by persistent high-frequency nerve stimulation. In contrast, the nonpeptide transmitters are synthesized enzymatically and stored in the axon terminal. Conservation of the nonpeptide transmitters is achieved by reuptake and storage into the nerve axon terminal. Thus, the duration of the postsynaptic response is dynamically regulated and depends on many factors, both pre-and postsynaptic, that modify transmitter synthesis, release, inactivation, and receptor sensitivity.

6. NERVE ACTIVITY CAN BE MEASURED BY DETERMINING THE FIRING RATE OR RATE OF NEUROTRANSMITTER RELEASE OR TURNOVER

One of the most important issues in neurobiology is determining the activity of a specific nerve or type of nerve under various physiologic or behavioral states. This challenge has been addressed primarily by electrophysiologic or neurochemical methods.

6.1. Nerve Activity Estimated by Electrical Behavior

Nerve activity is usually characterized by measuring the electrical behavior of the cell under resting or basal conditions and following the application of some stimulus. This can be done by inserting an electrode into the brain and measuring electrical activity under these different conditions. However, it is extremely difficult to make these measurements in an awake, freely moving animal, in which movement of the electrode by as much as a few micrometers can confound results. Refinement of electrophysiologic methods has progressed from extracellular recordings of nerve bundles to measurements of single neurons, and most recently to the technique of *patch clamping*. In this technique, a small patch of membrane from an individual neuron is sucked onto the end of a micropipette, and the electrical properties of this patch are studied. This technique is used to determine the types of ionic channels present on a small, isolated piece of membrane.

6.2. Nerve Activity Determined by the Rate of Transmitter Release or Turnover

Various techniques have been developed to measure the rate of release of a transmitter. These include monitoring the release from isolated neurons in tissue culture (*in vitro*), placing a small cannula (e.g., push-pull cannula) within a brain area of an anesthetized or awake animal and perfusing that area of the brain with physiologic media, or implanting a similar perfusion cannula attached to a dialysis membrane (e.g., microdialysis) that restricts the recovery of brain elements to only specific molecular weights. Transmitter release can then be determined in the media, perfusate, or dialysate.

Several turnover techniques have been used to measure neuronal activity. The rate of transmitter use can be estimated by measuring its disappearance after chemically inhibiting its synthesis. An example of this technique is the use of α -methyl *p*-tyrosine to inhibit tyrosine hydroxylase, the rate-limiting enzyme in the synthetic pathway for norepinephrine and dopamine, and then measuring the rate of disappearance of norepinephrine or dopamine. Turnover can also be estimated by measuring the rate of accumulation of the transmitter after chemically inhibiting its metabolic breakdown or by measuring the rate of disappearance of its metabolite. An example is the measurement of the appearance of serotonin or the disappearance of its primary metabolite, 5-hydroxyindoleacetic acid, after inhibiting the enzyme monoamine oxidase (MAO) with drugs such as pargyline and iproniazid.

All of these techniques have provided valuable information about the activity of specific neurotransmitter pathways under different physiologic conditions and behavioral states. They have led to the development of many behavioral models, such as the concept that the transmitter dopamine is involved in feelings of reward and reinforcement.

7. SMALL-MOLECULAR-WEIGHT NEUROTRANSMITTERS

The small-molecular-weight transmitters are made locally within the nerve-cell axon or dendrite. The well-established small-molecular-weight transmitters and modulators include three amino acids, five biogenic amines (essentially decarboxylate derivatives of amino acids), and acetylcholine. All of these molecules are synthesized and stored in the axon terminal, from which they are released after the arrival of an action potential. The response

produced by these transmitters can be fast or slow, depending on the specific type of postsynaptic receptor present. If their synthesis required axoplasmic transport from the cell body, they would soon be depleted. The enzymes responsible for making these compounds are synthesized in the cell body and transported to the axon, where the actual transmitter synthesis takes place (3).

The discussion of these transmitters focuses on the history of the discovery of the transmitter, the mechanism of their biosynthesis, secretion, and catabolism (e.g., degradation), their anatomic distribution, and their functions and physiologic actions. The function of many of these chemical messenger systems is still poorly understood. Our knowledge of these functions has often come from studying cases of behavioral or physiologic alterations caused by disease states, specific lesions, or the effects of pharmacologic intervention.

7.1. Acetylcholine Is the Prototypical Neurotransmitter

The first neurotransmitter to be isolated and identified was the small ester of the lipid choline, *acetylcholine*. The entire concept of neurotransmission developed from its discovery. In 1921 in Germany, Otto Loewi showed that a chemical substance, released on stimulation of the vagus nerve of an isolated frog's heart, would slow the beating of another frog's heart that was bathed in the same media. He called this substance *vagus-stuff* because it was released by stimulation of the vagus nerve. Nerve systems that use acetylcholine as a transmitter are called *cholinergic nerves*.

7.1.1. ANATOMY AND FUNCTION OF CHOLINERGIC NEURONS

Cholinergic neurons are found in both the central and peripheral nervous system. Peripheral nerves that use acetylcholine include several important pathways, including the motor neurons that begin in the ventral root of the spinal cord and innervate striated skeletal muscle; the preganglionic sympathetic and parasympathetic nerves that begin in the intermediolateral column of the spinal cord or brain stem and activate all the autonomic ganglia; the postganglionic parasympathetic nerves that innervate the viscera (e.g., heart, pulmonary bronchi, gastrointestinal tract, bladder, eye, and exocrine glands); and the postganglionic sympathetic cholinergic nerves to the major sweat glands of the skin. Because of their simplicity and the relative ease of studying them, much

has been learned about nerve transmission from these peripheral cholinergic nerves.

Although all of these pathways use the same neurotransmitter—acetylcholine—the postsynaptic receptors that trigger the cellular response are fundamentally different. The receptors located at the neuromuscular junction and the autonomic ganglia are known as *nicotinic* because they are activated specifically by the drug nicotine. In contrast, the receptors of the viscera (e.g., the gut, heart, and lungs) that are innervated by the parasympathetic nerves and the sweat glands are called *muscarinic* because they are activated by the compound muscarine and do not respond to nicotine. These different cholinergic receptors are also found in the brain, where the muscarinic types outnumber the nicotinic receptors by 10-fold to 100-fold.

Release of acetylcholine from spinal nerves is the initial signal from the central nervous system (CNS) in most peripheral response pathways. Acetylcholine release from somatic nerves triggers the contraction of skeletal muscle. In the autonomic ganglia, acetylcholine stimulates the postganglionic neurons that

form both the sympathetic and parasympathetic nerves. Acetylcholine released from parasympathetic nerves slows down the heart rate; constricts the smooth muscle of the bronchi, stomach, intestines, bladder, and eye; and stimulates secretion of various enzymes, hydrochloric acid, and mucus from exocrine glands. A cholinergic receptor on the endothelium of the vasculature induces a biochemical event that produces nitric oxide from the amino acid arginine, which causes smooth-muscle relaxation. Studies have shown that nitric oxide synthetase is present all over the brain, suggesting that nitric oxide may be a very important signaling system.

In the CNS, cholinergic pathways are present throughout the brain. The major cholinergic pathways are diagrammed in Fig. 2. In the ventral forebrain, cholinergic cell bodies from the septum, diagonal band of Broca, and basal nucleus send axons to innervate the hippocampus, interpeduncular nuclei, and neocortex, respectively. Cholinergic cell bodies from the tegmentum of the brain stem innervate the hypothalamus and thalamus. There are also short cholinergic interneurons within the striatum.

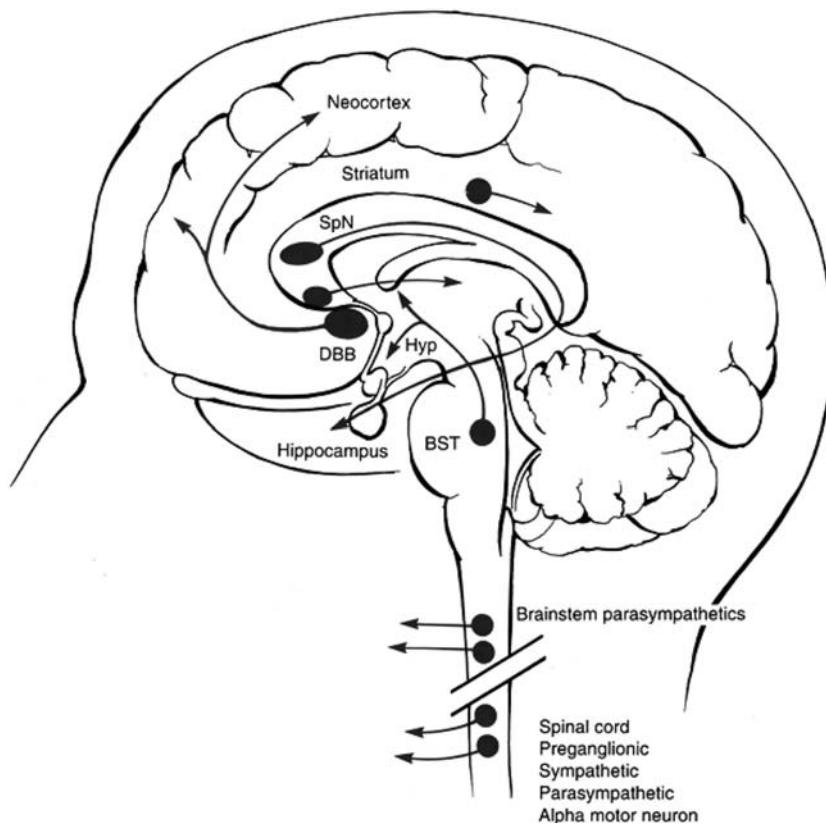


Fig. 2. Cholinergic pathways in the brain, brain stem, and spinal cord. In the brain, cholinergic cell groups are found in the septal nuclei (SpN), diagonal band of Broca (DBB), basal nucleus (BN), brain-stem tegmentum (BST), and small interneurons in the striatum. Autonomic and somatic motor nuclear groups are also found in the brain stem and spinal cord. Major projection areas are found in the frontal and parietal neocortex, hippocampus, hypothalamus (Hyp), and thalamus.

The central cholinergic pathways in the striatum play an important role in the central motor control of muscles. This is evidenced by the fact that benztropine, a drug that blocks muscarinic acetylcholine receptors, can alleviate the tremors associated with Parkinson's disease. Acetylcholine also seems to be important in memory consolidation, because cholinergic changes are found in the cortex of patients with Alzheimer's disease. Pharmacologic evidence suggests that blockade of the central muscarinic cholinergic synapses with scopolamine produces sedation and amnesia, whereas activation of nicotinic synapses increases alertness and is rewarding, as evidenced by cigarette smoking.

7.1.2. NEUROCHEMISTRY OF CHOLINERGIC NEURONS

The cholinergic axon has the capacity to synthesize, store, and secrete acetylcholine in a manner that is relatively independent from the nerve-cell body. The uptake of choline appears to be the rate-limiting step in acetylcholine synthesis.

Acetylcholine is synthesized from choline and acetylcoenzyme A in the nerve terminal by the enzyme choline acetyltransferase (Fig. 3). This enzyme, which has a molecular weight of about 67,000, is synthesized in the cell body and transported to the axon. The enzyme is a cytoplasmic enzyme, but because it is positively charged, it is often associated with intracellular, mitochondrial or vesicular membranes. Choline is a component of the complex lipids of all cell membranes occurring as phosphatidylcholine and sphingomyelin, and all cells have a choline uptake mechanism. However, uptake pumps in noncholinergic cells are of low affinity (K_m of 40 to 100 μM). Acetylcoenzyme A is produced within the nerve terminal itself, using normal energy sources. Acetylcholine is synthesized in the cytoplasm and subsequently sequestered in small electron-opaque granules that are most prevalent in the active zone of the nerve terminal. Acetylcholine is stored within these granules with adenosine 5' triphosphate (ATP) at a concentration of about 1 M and with a charged anion protein known as vesiculin.

When acetylcholine is released, it is rapidly degraded by acetylcholinesterase, an enzyme that is found in high concentrations within the synaptic cleft and in other areas. True acetylcholinesterase is found in the synapse and is associated with the neuromuscular junction and cholinergic areas of the brain. This enzyme degrades acetylcholine to choline and acetic acid and is one of the fastest enzymes known. The maximal velocity of acetylcholine degradation has been measured at 75 g of substrate per hour using

Acetylcholine Synthesis and Degradation

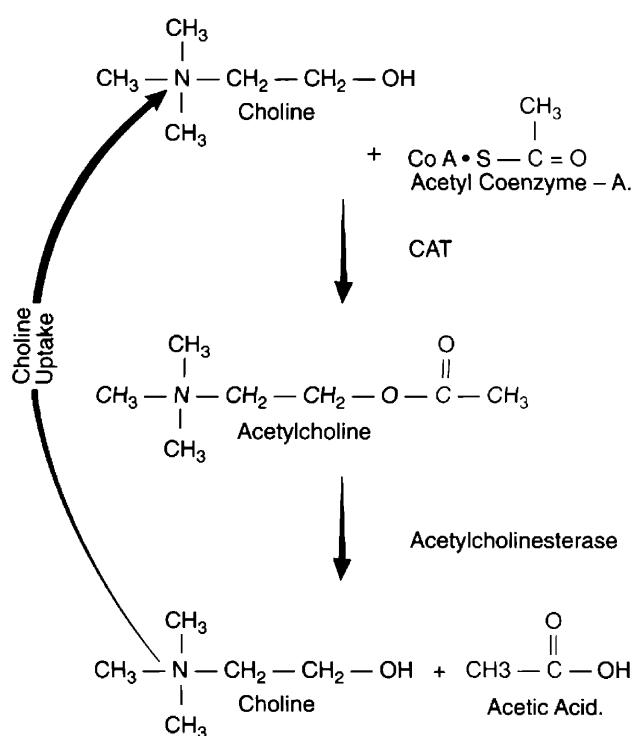


Fig. 3. Acetylcholine synthesis is catalyzed in the cytoplasm of cholinergic cells by the enzyme choline acetyltransferase (CAT). The transmitter is degraded outside of the cell by the enzyme acetylcholinesterase into choline and acetic acid. Choline is taken back up in the nerve by a high-affinity pump for reuse.

1 mg of purified enzyme. It has been calculated that 1 molecule of cholinesterase can hydrolyze as many as 5000 molecules of acetylcholine per second. These biochemical events are diagrammed in Fig. 3. Acetylcholine can also be metabolized by pseudo- or butyrylcholinesterase, which is found primarily in plasma.

The importance of acetylcholinesterase for the termination of cholinergic transmission can be demonstrated if this process is pharmacologically inhibited. Many drugs, insecticides, and even chemical warfare agents, such as sarin and soman, inhibit acetylcholinesterase in a slowly reversible or irreversible fashion. The pharmacologic actions of these compounds produce overstimulation of the cholinergic receptor, which can lead to tetanic paralysis and death.

The choline that is formed from the degradation of acetylcholine is actively pumped back into the nerve terminal and reused to synthesize more acetylcholine. To accomplish this reuptake, the cholinergic neuron

has developed a high-affinity choline pump (K_m of 0.4 to 4.0 μM) that rapidly sequesters any choline from the synaptic cleft. This process for supplying choline for transmitter synthesis appears to be the *rate-limiting step* for the production of this transmitter. Drugs that inhibit the uptake of choline (such as hemicholinium) produce rapid paralysis of muscular activity. The muscles that are used most are those that become paralyzed first, indicating that the rate of cholinergic-nerve activity in these muscles leads to exhaustion and depletion of acetylcholine.

7.2. Biogenic Amine Transmitters

The aminergic transmitter systems have been given several names, which can cause confusion. The biogenic amines that are synthesized from tyrosine are known as *catecholamines*, because their structure contains a catechol moiety (e.g., a phenol ring with two hydroxyls). They are also referred to as adrenergic after the English term, *adrenaline*. Serotonin, or 5-hydroxytryptamine (5-HT), is also classed as a biogenic amine. Structurally, however, it is an indolamine because its ring structure arises from tryptophan. Histamine is a biogenic amine that is present in the brain and the enteric nervous system of the gastrointestinal tract. Chemically, it is the decarboxylated amino acid histidine, and it is present in high concentrations in mast cells and in some neuronal pathways.

7.2.1. CATECHOLAMINE TRANSMITTERS

The catecholamine transmitters norepinephrine, epinephrine, and dopamine serve a variety of functions in the peripheral and central nervous system. Around the beginning of the 20th century, when acetylcholine was being established as a neurotransmitter, the sympathetic nerves and the adrenal medulla were being extracted for substances that raised the blood pressure and accelerated the heart rate. Otto Loewi, one of the discoverers of acetylcholine, also worked with the sympathetic transmitter and called it *accelerant-stuff* because it increased the heart rate. The adrenal medulla is an autonomic ganglia where the postganglionic neurons (designated chromaffin cells) do not possess long axons. They have the capacity, as do a variety of neurons in the brain, to synthesize the catecholamine transmitter and neurohormone, known as epinephrine.

When exposed to light or alkaline pH, catecholamines oxidize to colored substances known as quinones. This helped to establish the chemical identity of these amines and helped identify some of their pathways in the brain. The *substantia nigra* of the

midbrain, for example, is named with the Latin term for “black substance.” This area of the brain, which contains dopamine, turns dark when exposed to light or air. In patients with Parkinson’s disease, the dopamine-producing neurons of the substantia nigra that send axons to the striatum die off, leading to the clinical manifestations of this disease.

The catecholamines serve as sympathetic nervous system transmitters and help to control most visceral activity. The major amine neurotransmitter is norepinephrine, and epinephrine and norepinephrine are secreted in a ratio of 4:1 from the adrenal medulla. Dopamine may also be an unrealized sympathetic neurotransmitter, particularly because specific dopamine receptors have potent actions on kidney blood flow.

The peripheral actions of the catecholamines are well understood, because this is such an isolated system. The actions include constriction or dilation of the arteries that control blood pressure, the increase of heart rate and strength, dilation of the bronchioles of the lungs, a decrease in gastrointestinal activity, dilation of the iris of the eye, and a variety of metabolic effects, including glycogenolysis, lipolysis, and the release of renin.

The central actions of catecholamines can best be described as arousal. Because of the complexity of the brain, their functions are not fully elucidated, but they can be partially understood from experiments using specific lesions of catecholamine pathways or the actions of selective drugs.

7.2.2. CATECHOLAMINE SYNTHESIS

The amino acid tyrosine is the common precursor for all three catecholamine transmitters—dopamine, norepinephrine, and epinephrine. The biosynthesis of the catecholamine neurotransmitters occurs primarily in the nerve terminal, as for other small-molecular-weight transmitters. The enzymes that catalyze this synthesis are made and packaged in the nerve-cell body, where they are synthesized by ribosomes before transport down the axon to the terminal.

Within the terminal, specific membrane pumps supply tyrosine or phenylalanine as the precursors for amine biosynthesis. The details of this pathway are shown in Fig. 4. Some of the biochemical parameters of the enzymes that synthesize the transmitters are also shown in Table 3. Tyrosine hydroxylase, the first and rate-limiting enzyme for the synthesis of all three catecholamines, is found in the cytoplasm. This enzyme requires molecular oxygen, iron, and a cofactor known as tetrahydrobiopterin. This cofactor helps to maintain tyrosine hydroxylase in a reduced,

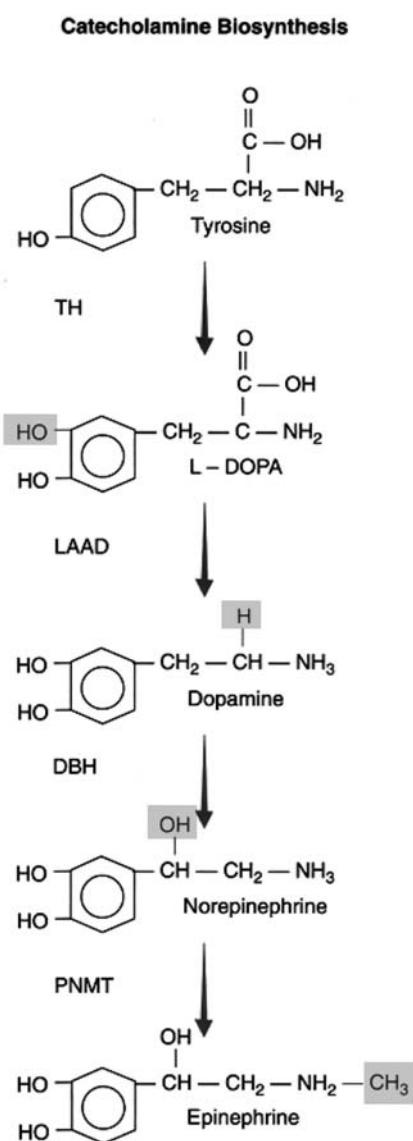


Fig. 4. The biosynthesis of the catecholamines norepinephrine, epinephrine, and dopamine from the common precursor, tyrosine. The enzymes involved are tyrosine hydroxylase (TH), L-amino acid decarboxylase (LAAD), dopamine-β-hydroxylase (DBH), and phenylethanolamine-N-methyltransferase (PNMT). The structural changes at each step are shaded.

active state. When catecholamines, such as dopamine or norepinephrine, build up in the cytoplasm, they inhibit the ability of the pteridine to activate tyrosine hydroxylase. This end-product inhibition provides one of the major regulatory steps in catecholamine synthesis. Other important regulatory steps include the phosphorylation of tyrosine hydroxylase, which increases its affinity for the pteridine cofactor. This allosteric activation appears to be mediated by a variety of presynaptic receptors that use cyclic

3',5'-AMP or intracellular calcium concentration as second messengers. Thus, catecholamine synthesis is controlled by the rate-limiting enzyme tyrosine hydroxylase, which can be regulated by end-product feedback inhibition or by allosteric activation through second-messenger systems.

Hydroxylation of tyrosine by tyrosine hydroxylase yields the amino acid L-DOPA (L-3,4-dihydroxy-phenylalanine). This intermediate never reaches high concentrations in the cytoplasm because it is immediately decarboxylated by the enzyme L-amino acid decarboxylase. L-Amino acid decarboxylase and dopamine-β-hydroxylase have activities that are 10 to 1000 times higher than that of tyrosine hydroxylase. The activity of these enzymes is not directly proportional to the affinity (K_m) but depends on the amount of enzyme present and on cofactor regulation (Table 3). L-Amino acid decarboxylase is very fast, uses the cofactor pyridoxal phosphate (e.g., vitamin B₆), and yields dopamine, the first of the biogenic amine transmitters. Although dopamine was originally believed to be only an intermediate in norepinephrine synthesis, it is the major catecholamine transmitter in the mammalian brain, is found in the autonomic ganglia, and is a neurohormone in the hypothalamus, controlling pituitary prolactin secretion. In dopaminergic nerves, the transmitter is stored within secretory granules.

In noradrenergic nerves, dopamine is sequestered into secretory granules that contain the enzyme dopamine β-hydroxylase. This enzyme hydroxylates dopamine on the β-carbon atom by using the cofactors ascorbate (e.g., vitamin C), molecular oxygen (O₂), and copper, producing norepinephrine. The inside of these granules are relatively acidic, which is ideal for the pH maximum of this enzyme. Many of the peptidergic transmitters are amidated on their carboxyl-terminal ends by another vesicular enzyme that has identical pH and cofactor requirements. Noradrenergic nerves release the contents of these vesicles on stimulation. This type of nerve makes up the majority of sympathetic nerves in the periphery and many pathways in the brain. It is estimated that 10,000 to 15,000 transmitter molecules are stored in a single granule as a salt with calcium, ATP, and a protein known as chromogranin.

In adrenergic neurons in the brain or in the adrenal medulla, norepinephrine diffuses out of the secretory granules into the cytosol, where it is methylated by the last synthetic enzyme, called phenylethanolamine-N-methyltransferase (PNMT). This enzyme uses S-adenosylmethionine as a cofactor to add a

Table 3
Biochemical Properties of Enzymes

Enzyme	Molecular weight	Cofactors	Affinity, $K_m(M)$
Tyrosine hydroxylase	60,000	Tetrahydrobiopterin, molecular O_2 , Fe, NADPH	0.4×10^{-4} to 2×10^{-4}
L-Amino acid decarboxylase	85,000–90,000	Pyridoxal phosphate (vitamin B ₆)	4×10^{-4} (L-DOPA)
Dopamine-β-hydroxylase	75,000	Ascorbate (vitamin C), molecular O_2 , Cu ²⁺	5×10^{-3} (dopamine)
Phenylethanolamine-N-methyl transferase		S-Adenosylmethionine	
Tryptophan hydroxylase	60,000	Tetrahydrobiopterin, molecular O_2	5×10^{-5} (tryptophan)
Choline acetyltransferase	67,000	Acetylcoenzyme A	7.5×10^{-4} (choline) 1×10^{-5} (coA)
Glutamic acid decarboxylase	85,000	Pyridoxal phosphate (vitamin B ₆)	7×10^{-4} (glut) 5×10^{-5} (Vit. B ₆)

methyl group to the amino side of norepinephrine. The product, epinephrine (called adrenaline by the British), is repackaged into secretory granules that contain ATP, the protein chromogranin, and dopamine-β-hydroxylase. The epinephrine-to-ATP ratio in these granules is about 4:1 in the adrenal medulla.

The activity of PNMT can be upregulated by the adrenal steroid known as cortisone. A specific portal vascular system between the adrenal cortex and medulla facilitates this activation. A diurnal early-morning increase in cortisol secretion activates PNMT to facilitate epinephrine synthesis. The resulting increase in epinephrine secretion stimulates liver glycogenolysis, gluconeogenesis, and prepares mammals for the upcoming daily activity.

7.2.3. CATECHOLAMINE METABOLISM

Termination of catecholaminergic transmission is accomplished by active uptake of the released amines into the axon terminal, which are recycled or degraded enzymatically. Although the termination of cholinergic neurotransmission is accomplished exclusively by the enzymatic degradation of acetylcholine, termination of catecholaminergic transmission occurs primarily by active reuptake of the amines into the axon terminal.

Most cells, including platelets, appear to have some ability to pump catecholamines across their membranes. Catecholaminergic neurons have a very active high-affinity pump that sequesters the amines back into the axon before they can diffuse away from the synapse. This uptake, like that of choline, is sodium-dependent and has a K_m of 1 to 5 μM . The importance of this system for terminating aminergic

transmission is demonstrated by the action of the stimulant drug cocaine, which inhibits uptake of the amines and increases their availability for the post-synaptic receptors. The norepinephrine-transporter protein has been cloned and found to be a sodium-dependent membrane pump that is similar to the GABA transporter. It has 617 amino acids and contains 12 hydrophobic (or lipophilic) amino acid sequences that span the lipid membrane.

The two enzymes that degrade the catecholamines are catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO). The biochemical action of these enzymes on the amine structure is shown in Fig. 5. Although COMT attaches a methyl group to the ring, MAO oxidizes the amine part of the molecule. The catecholamines can be acted on by both enzymes, forming compounds that are methylated and oxidized. These metabolites are often measured in the urine to evaluate sympathetic activity or the presence of an adrenal tumor known as a pheochromocytoma.

MAO is an intracellular enzyme found in the mitochondrial membranes of neurons and glial cells of the brain and in the liver, kidney, glandular tissues, and intestines. Its wide distribution suggests its importance in metabolizing other compounds. For example, when MAO is pharmacologically inhibited for treating depressive illnesses, tyramine—a normally innocuous substance found in aged cheeses and wines—becomes a potentially lethal toxicant. When tyramine, which is normally oxidized by MAO, is absorbed intact from the gastrointestinal tract, it can cause the release of sympathetic transmitter, leading to hypertensive crisis and death.

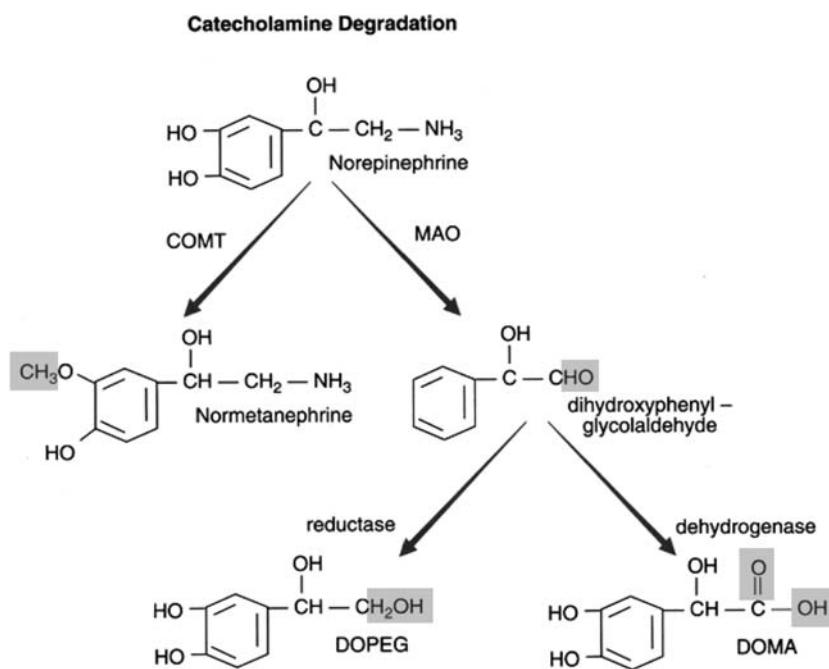


Fig. 5. Changes induced by the degradative enzymes catechol-*O*-methyl transferase (COMT) and monoamine oxidase (MAO) on catecholamine structure. The intermediate product of MAO is an aldehyde that can be further metabolized by aldehyde reductase to 3,4-dihydroxyphenyl-ethyl-glycol (DOPEG) or by aldehyde dehydrogenase to 3,4-dihydro-mandelic acid (DOMA). The structural changes at each step are shaded.

Monoamine oxidase has a molecular weight of about 102,000 Da and occurs in two forms, MAO-A and MAO-B, based on substrate specificity and pharmacologic inhibition. Nonselective MAO inhibitors are useful in the treatment of depressive illness, apparently because of their ability to make more biogenic amines available in the brain areas responsible for controlling mood. A selective inhibitor of MAO-B known as selegiline is used in treating Parkinson's disease, as dopamine is primarily metabolized by MAO-B.

MAO oxidizes the amines to their corresponding aldehydes. As shown in Fig. 6 and Fig. 7, the aldehyde product of norepinephrine and epinephrine can then be acted on by aldehyde dehydrogenase or aldehyde reductase to produce dihydroxyphenylethylglycol (DOPEG) or dihydroxymandelic acid, respectively. These can then be acted on by COMT to produce the 3-methoxy derivatives, 3-methoxy-4-hydroxy-phenylethylglycol (MHPG) and vanillylmandelic acid (VMA), respectively. The urinary excretion of these metabolic products, particularly VMA, is often used in the diagnosis of autonomic nervous system disorders. Because the aldehyde reductase pathway is believed to be more active in the CNS, MHPG or DOPEG would be the products of increased central noradrenergic and adrenergic activity.

Catecholamine Degradation

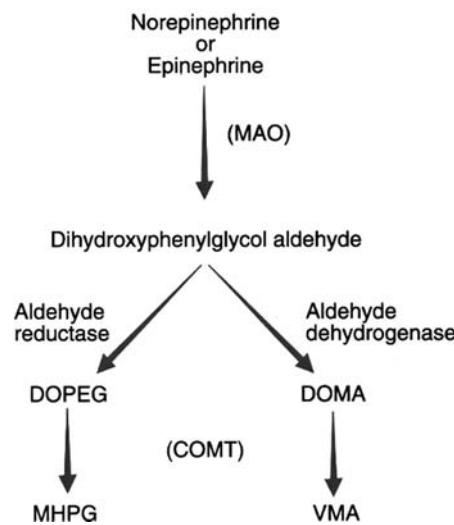


Fig. 6. Monoamine oxidase (MAO), aldehyde reductase, aldehyde dehydrogenase, and catechol-*O*-methyl transferase (COMT) catalyze the degradation of norepinephrine and epinephrine. The products include 3,4-dihydroxyphenylethylglycol (DOPEG), 3,4-dihydro-mandelic acid (DOMA), 3-methoxy-4-hydroxyphenylethylglycol (MHPG), and vanillylmandelic acid (VMA).

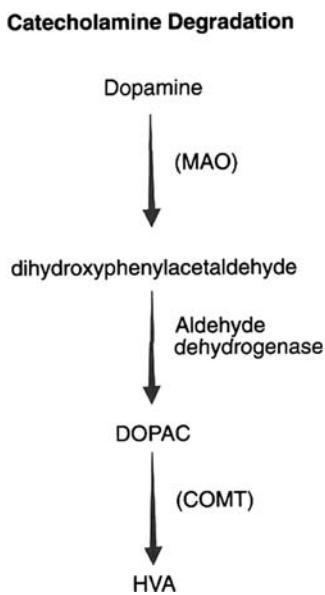


Fig. 7. The enzymatic degradation of dopamine is catalyzed by monoamine oxidase (MAO) and catechol-*O*-methyl transferase (COMT). The major products are 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA).

The primary MAO metabolite of central dopamine is dihydroxyphenylacetic acid (Fig. 7), which can be further metabolized by COMT to produce homovanillic acid. These metabolites are often used in research as a measure of noradrenergic or dopaminergic activity in the brain, and they may soon become clinically important.

COMT is a cytoplasmic enzyme that is distributed even more widely than is MAO. It is found in high concentrations in the kidney and liver. The enzyme has a molecular weight of about 24,000 Da and requires *S*-adenosylmethionine and the divalent cation, Mg^{2+} . The enzyme transfers a methyl group from *S*-adenosylmethionine to the 3-hydroxy group of the catecholamine. It can also methylate catechol-based drugs such as isoproterenol, a β -adrenergic receptor stimulating drug that is useful in treating asthma. Epinephrine, norepinephrine, and dopamine are methylated by COMT to produce metanephrine, normetanephrine, and 3-methoxytyramine, respectively. Measuring the amounts of these compounds excreted in the urine is useful in the diagnosis of the adrenal tumor pheochromocytoma.

7.2.4. CATECHOLAMINE PATHWAYS

Specific catecholamine pathways have been mapped throughout the brain and appear to function in a variety of behavioral, cognitive, and physiologic processes. The three catecholaminergic pathways can

be visualized by techniques known as fluorescent microscopy and immunocytochemistry.

Fluorescent histochemistry uses the property of the catechol molecules to fluoresce when exposed to chemicals such as formaldehyde. This method was initially used to map the catecholamine and indolamine (e.g., serotonin) pathways within the CNS. The drawback of this technique is that it is difficult to differentiate various catecholaminergic or indolaminergic pathways because of the similar wavelengths of emitted light.

Immunocytochemistry is a method that uses specific antibodies that are generated against the purified enzymes involved in catecholamine biosynthesis. The antibodies bind to the enzymes (or specific transmitters) that are fixed on histologic sections of the brain. This technique can be used to label the cell bodies, axons, and terminals of a specific neuronal pathway. The bound antibodies are detected by using chemical reactions that produce a color change or electron-opaque product in the neurons containing the antigen. In practice, if a pathway contains the enzyme PNMT, which methylates norepinephrine to epinephrine, it can be assumed that this pathway is adrenergic, although it would also contain dopamine- β -hydroxylase and tyrosine hydroxylase. If the pathway only contains dopamine- β -hydroxylase and tyrosine hydroxylase, it would probably be *noradrenergic*, but one that contains only tyrosine hydroxylase would be *dopaminergic*.

Dopamine is the major catecholamine neurotransmitter in the mammalian CNS. Dopamine was originally believed to be only a precursor in the synthesis of norepinephrine and epinephrine, but it was discovered that dopamine comprised as much as 50% of the total catecholamines in the brain of mammals. Moreover, the localization of dopamine and norepinephrine within the brain did not always coincide. The dopaminergic neuronal systems are heterogeneous and have been classified by the Swedish investigators who developed the fluorescent technique into several specific nuclear groups designated A8–A17.

Dopamine is found in interneurons in the peripheral autonomic ganglia. Similar dopaminergic interneurons with very short axons are also found in the retina and in the olfactory bulb, where they appear to modify sensory input through inhibition of their target neurons. In the retina, this is called lateral inhibition, which is important for processing visual information.

Dopaminergic neurons with intermediate-length axons include the tuberoinfundibular and hypophysial,

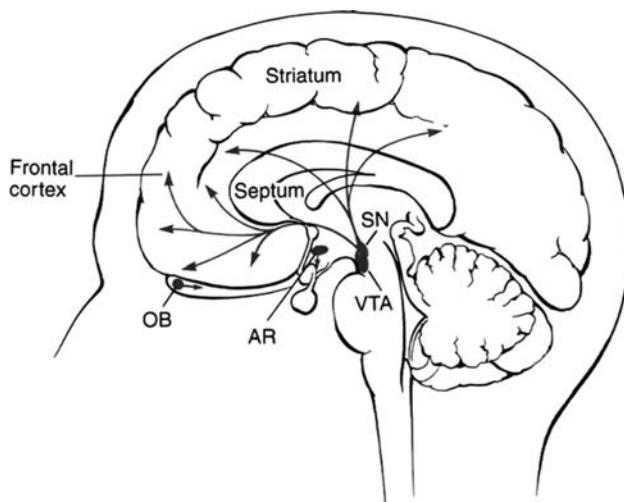


Fig. 8. The major dopaminergic pathways in the CNS. Dopaminergic cell groups from the substantia nigra (SN) project to the caudate and putamen. Cell bodies near the substantia nigra in the ventral tegmental area (VTA) project axons to the septum, limbic cortex (including the frontal and cingulate cortex), amygdala, nucleus accumbens (NA), and olfactory tubercle (OB). Other discrete dopaminergic systems exist in the hypothalamic arcuate (AR) and periventricular nuclei, in the olfactory bulb, and in the retina.

incertohypothalamic cells and the medullary periventricular group. The tuberoinfundibular neurons have a neurohumoral function; they secrete dopamine into a portal vascular system that supplies the anterior pituitary. This dopamine is responsible for inhibiting secretion of the anterior pituitary hormone, prolactin. Many of the dopaminergic pathways in the brain are diagrammed in Fig. 8.

The final subdivision of dopaminergic neurons includes the midbrain groups from the substantia nigra and the ventral tegmental area. These systems have long axons that innervate the basal ganglia, parts of the limbic system, and the frontal cortex. The neostriatal system, which has cell bodies in the substantia nigra, innervates the caudate and putamen. This suggests that dopamine released from neostriatal areas has motor functions. The motor problems associated with Parkinson's disease are caused by a decrease in dopamine in these areas. Administration of the dopamine precursor L-DOPA bypasses tyrosine hydroxylase and alleviates some of the motor disturbances of Parkinson's disease.

The specificity and complexity of the dopaminergic systems is further demonstrated by the mesolimbic system. These neurons originate in the ventral tegmental area of the midbrain, next to the substantia

nigra. Long axons from these neurons project to many parts of the limbic system, including the nucleus accumbens, olfactory tubercle, septum, amygdala, and limbic cortex (e.g., frontal and cingulate cortex). These areas are associated with mood alterations and cognitive function, indicating another important role of central dopamine. The nucleus accumbens is involved with reward, and the release of dopamine in this area generates positive feelings of reinforcement. It is in this area that the stimulant properties of cocaine and amphetamine (which releases axonal dopamine) are believed to act. The actions of many antidepressant drugs may also be associated with these brain areas. These agents, which inhibit MAO or the amine uptake pump, increase the amount of dopamine available for the dopamine receptor in these areas. The noradrenergic and serotonergic systems may also be involved in mood disorders.

The role of dopamine in cognition can be demonstrated by the action of a group of drugs used in treating schizophrenia. These agents, known as neuroleptics, block dopamine receptors and alleviate many of the hallucinations and ideations associated with this disease. However, prolonged therapy with some dopamine-blocking drugs can adversely affect the nigrostriatal system and produce a variety of adverse effects, including a Parkinson-like syndrome and an abnormal involuntary-movement syndrome called tardive dyskinesia.

The *noradrenergic pathways* of the brain stem are highly diffuse, project all over the brain, and are probably involved in activation of cognitive function and mood. The noradrenergic system in the brain comprises far fewer cells and is less specific than the dopaminergic system. Although there are 30,000 to 40,000 dopamine cells in the midbrain system alone, there are only about 10,000 noradrenergic neurons in the entire brain, which are localized in the brain stem. Specifically, noradrenergic cell bodies are found in the locus coeruleus and lateral tegmental nuclei of the brainstem (Fig. 9). *Coeruleus* means "blue" and refers to the pigment associated with this area. Although there are very few cells in these nuclei, the axons are highly branched and project to all parts of the CNS. Noradrenergic axons can be found throughout the cortex, limbic system, hypothalamus, olfactory bulb, cerebellum, medulla, and spinal cord.

Instead of forming discrete synapses at the ends of axonal branches, noradrenergic neurons elaborate varicosities all along their axons that contain norepinephrine-filled vesicles. These are known as

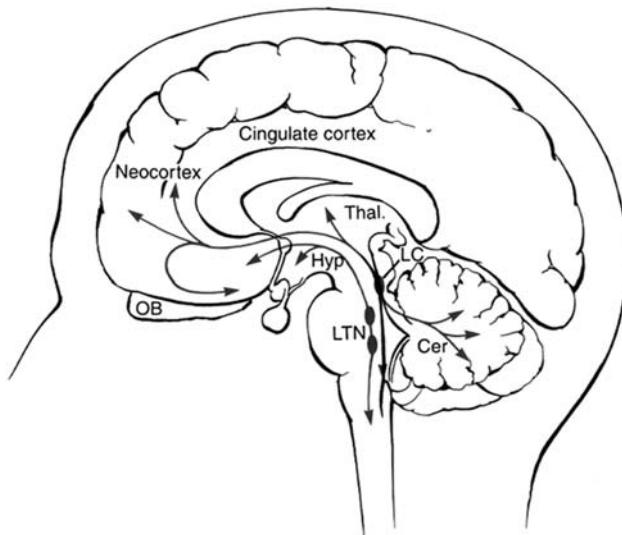


Fig. 9. The noradrenergic pathways in the CNS are restricted to several cell groups in the brain stem. The limited number of noradrenergic cell bodies in the locus coeruleus (LC) and the lateral tegmental nuclei (LTN) send highly branched axons to all areas of the brain, including the neocortex, cingulate cortex, thalamus (Thal.), hypothalamus (Hyp.), olfactory bulb (OB), and cerebellum (Cer).

diffuse synapses and resemble a string of beads or pearls when viewed by fluorescent microscopy. Presumably, the transmitter released at this type of synapse bathes the postsynaptic target area to initiate a response. These CNS noradrenergic neurons closely resemble the noradrenergic neurons found in the sympathetic nervous system. In contrast with the noradrenergic diffuse synapses, *contact synapses*, in which specific synaptic processes are formed at the end of the axons, are typical of most other transmitter systems, as exemplified by the neuromuscular junction.

Noradrenergic neurons, such as dopaminergic neurons, can inhibit or excite the postsynaptic cells, depending on which receptor subtype is present. Within the CNS, many neurons have been found to express β -adrenergic receptors, which use cyclic AMP as a second messenger. Within the locus coeruleus, pharmacologic studies have shown that α_2 -adrenergic receptors influence firing rates. Noradrenergic activity inhibits spontaneous firing of large portions of the CNS and may enhance signal-to-noise levels in the brain.

The noradrenergic system appears to be important in arousal or motivation. Stimulant drugs, such as cocaine and amphetamine, and some tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) increase the amount of norepinephrine

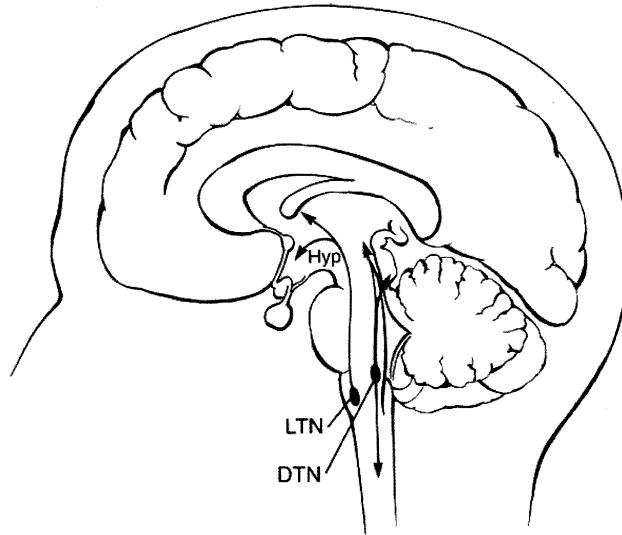


Fig. 10. The adrenergic pathways in the brain originate from a few cell groups in the lateral (LTN) and dorsal tegmental nuclei (DTN). These cell bodies send axons to the hypothalamus (HYP), locus coeruleus (LC), and to the intermediolateral-cell columns of the spinal cord.

available for binding to adrenergic receptors, potentiating the noradrenergic system. Most studies suggest that increased noradrenergic activity is associated with heightened vigilance and behavioral awareness and that decreased noradrenergic activity results in more vegetative processes. Central norepinephrine has also been implicated in pain pathways, memory, and the control of autonomic and endocrine function. The distribution of the noradrenergic neurons in the brain is diagrammed in Fig. 9.

Epinephrine-containing neurons in the brain are restricted to the brain stem and may be important in regulating autonomic and endocrine function. Adrenergic neurons in the brain stem have been visualized by staining for their unique enzyme, PNMT. Figure 10 shows the distribution of known adrenergic neurons in the brain. These cells are restricted to nuclei in the lateral and dorsal tegmentum (designated Cl-3 by the Swedish group that initially described them). They ascend to innervate the hypothalamus and descend the spinal cord to innervate the intermediolateral-cell column that gives rise to the preganglionic nerve cell bodies of the sympathetic nervous system.

Little is known about the function of epinephrine in the brain, although it does inhibit firing of neurons in the locus coeruleus. Based on epinephrine's anatomic distribution, it seems to be important in

the regulation of autonomic and neuroendocrine hypothalamic function. Many studies attest to this supposition.

7.3. Serotonin

Serotonin is an indolamine present in neural pathways that parallel the distribution of norepinephrine in the brain. Serotonin is found in many cells of the body, such as blood platelets, mast cells, and the enterochromaffin cells of the gastrointestinal tract, and in brain cells. It was originally discovered in blood, where it has a vasoconstrictor effect on the arteries. For this reason, it was believed to be the cause of high blood pressure. So much serotonin exists in peripheral platelets, mast cells, and the gastrointestinal tract that the amount in the brain only represents 1% to 2% of the total amount found in the body. Serotonin is also found in the pineal body of the brain, where it functions as the precursor for melatonin, an indolamine that is considered important in circadian cycles and reproductive function.

7.3.1. SEROTONIN SYNTHESIS

Serotonin is synthesized from the essential amino acid tryptophan, which crosses the blood-brain barrier by a mechanism that also supplies the brain with aromatic and branched-chain amino acids. The concentration of serotonin in the brain is sensitive to the availability of tryptophan in the diet, so that brain serotonin levels can be modified by competition for uptake by other amino acids. In the serotonergic neuron, tryptophan is hydroxylated to 5-hydroxytryptophan (5-HTP) by an enzyme known as tryptophan hydroxylase (Fig. 11). This enzyme is similar to tyrosine hydroxylase because both use molecular oxygen and a tetrahydropteridine cofactor. Like tyrosine hydroxylase, it is also regulated by phosphorylation, calcium, phospholipids, and partial proteolysis.

The genes for many hydroxylase enzymes have now been cloned and characterized, making it possible to compare their amino acid sequences. These studies show that tyrosine, tryptophan, and phenylalanine hydroxylases probably originated or evolved from a common ancestral gene. The catalytic and regulatory elements of all three enzymes show marked similarity, but there are also differences.

Unlike tyrosine hydroxylase, tryptophan hydroxylase is not regulated by end-product inhibition. Although dopamine or norepinephrine can feed back to inhibit their own synthesis, increased cytoplasmic serotonin does not inhibit tryptophan hydroxylase. For example, if an inhibitor of MAO (an enzyme that degrades

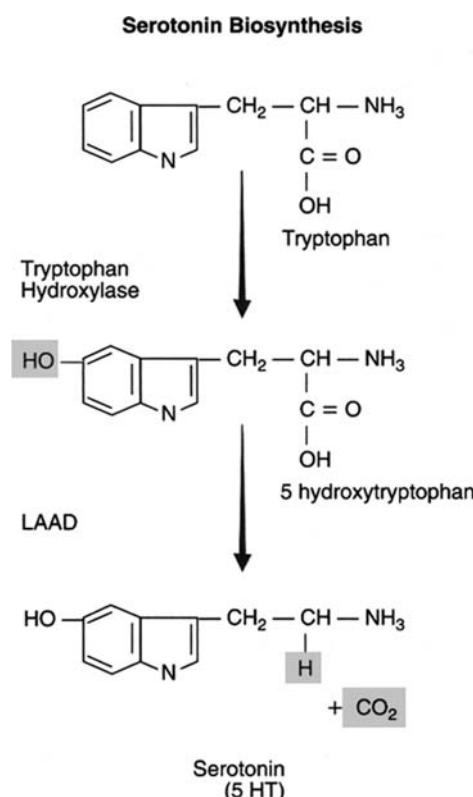


Fig. 11. Serotonin biosynthesis begins with the hydroxylation of tryptophan to 5-hydroxytryptophan by tryptophan hydroxylase. This amino acid is decarboxylated by L-amino acid decarboxylase (LAAD) to serotonin (5-HT). The shaded groups represent enzymatic additions or deletions.

catecholamines and serotonin) is administered, the concentration of serotonin in the brain increases by 300%. This is not the case with the catecholamines.

The second enzymatic step in the formation of serotonin is the decarboxylation of 5-HTP. This is accomplished by the aromatic amino acid decarboxylase, an enzyme that also decarboxylates L-DOPA to produce dopamine. Because this enzyme has a very high activity, it is not rate-limiting in the synthesis of serotonin, and therefore little 5-HTP is expected to be present in these neurons.

What regulates serotonin turnover? Several factors are probably involved. Presynaptic neuromodulation can regulate tryptophan hydroxylase activity by activation of second-messenger systems that phosphorylate and activate the enzyme. The K_m for tryptophan hydroxylase (Table 3) is higher than the amount of tryptophan in the blood. This means that the enzyme is not saturated, and that the availability of substrate may also regulate activity. This suggests that in some circumstances, nutrition and diet can alter brain activity and mood.

As in the case of the catecholamines, serotonin is actively taken up and stored in a specific set of secretory granules. The pumping mechanism responsible for the uptake of serotonin (and the catecholamines) into these granules is inhibited by the drug reserpine. If the storage of the amines is inhibited by this drug, all four of the biogenic amines are depleted. Because of this action, this plant alkaloid has been used for centuries as a sedative, in psychosis, and for hypertension.

Once serotonin is released into the synaptic cleft, the serotonergic response is terminated by reuptake into the presynaptic axon terminal. An important class of antidepressant drugs, known as selective serotonin reuptake inhibitors (SSRIs), blocks the reuptake of serotonin from the synaptic cleft, thus prolonging and strengthening the postsynaptic response. Fluoxetine (Prozac), sertraline (Zoloft), and paroxetine (Paxil) are examples of SSRIs.

In the pineal gland, serotonin undergoes two additional biochemical steps to produce the pineal “hormone” melatonin. First, serotonin is *N*-acetylated to make *N*-acetyl serotonin followed by methylation of the hydroxy group to melatonin by the enzyme 5-hydroxyindole-*O*-methyltransferase. The first enzyme in this pathway, *N*-acetyltransferase, is subject to regulation by the sympathetic nervous system-mediated β -adrenergic receptor, which alters cyclic AMP formation during the day-night cycle.

7.3.2. SEROTONIN METABOLISM

The major pathways for the degradation of serotonin are reuptake into the nerve and degradation by MAO. There are many similarities between the catecholaminergic and indolaminergic metabolic pathways. Unlike the catecholamines, indolamines cannot be degraded by methylation by COMT because of the difference in the ring structure and the presence of only one hydroxyl on the phenolic ring. The pathway for serotonin degradation is shown on Fig. 12. MAO oxidizes serotonin to 5-hydroxyindoleacetic acid through an indoleacetaldehyde intermediate or 5-hydroxytryptophol through an aldehyde reductase, depending on the NAD^+/NADH ratio in the brain.

The similarities between the uptake and degradation processes are such that many of the same drugs that inhibit catecholamine uptake and MAO degradation do the same for serotonin. For example, many of the tricyclic antidepressants also inhibit neuronal serotonin uptake, and all of the MAO inhibitors inhibit serotonin degradation. Together with the SSRIs, these drugs are useful in the treatment of

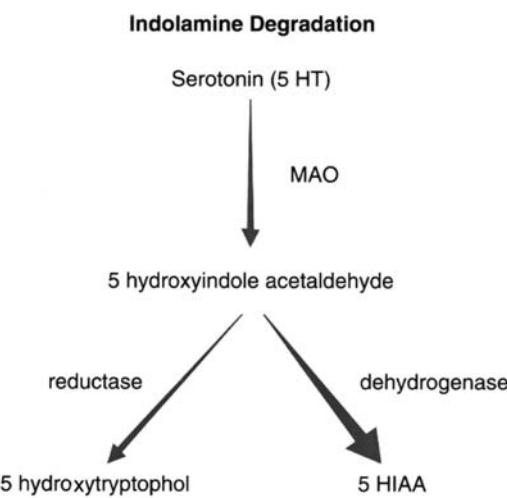


Fig. 12. Serotonin degradation is enzymatically catalyzed in a manner similar to that for the catecholamines. Serotonin or 5-hydroxytryptamine (5-HT) is acted on by monoamine oxidase (MAO) to yield 5-hydroxyindole acetaldehyde. This intermediate is metabolized by an aldehyde reductase or dehydrogenase to yield 5-hydroxytryptophol or 5-hydroxyindole acetic acid (5-HIAA).

affective disorders such as anxiety and depression, a topic that is further discussed in the section on the aminergic theory of the affective disorders.

7.3.3. SEROTONIN PATHWAYS

The *serotonergic pathways* originate in the raphe nucleus, area postrema (AP), and caudal locus coeruleus and generally parallel the noradrenergic pathways. Although the mapping of the serotonergic systems of the brain was initially hindered by some of the limitations of the histofluorescent microscopy, these systems are now well-delineated anatomically. The cell bodies of the serotonergic nerves in the brain arise from several groups of cells in a midline area of the pons and upper brain-stem called the *raphe nuclei* (Fig. 13). They have been classified as the B1 to B9 serotonergic nerve groups. The more rostral of the nuclei innervate the cortex, thalamus, and limbic systems, and the posterior nuclei (e.g., B1, B2, and B3) innervate the medulla and spinal cord. Unlike the organized nuclear distribution of the dopaminergic system, the serotonergic nuclei innervate much of the telencephalon and diencephalon in an overlapping manner.

The major functions of the serotonergic system remain relatively ill-defined. More than 90% of the brain serotonin can be depleted with *p*-chlorophenylalanine, a drug that inhibits tryptophan hydroxylase, with few gross effects on animal

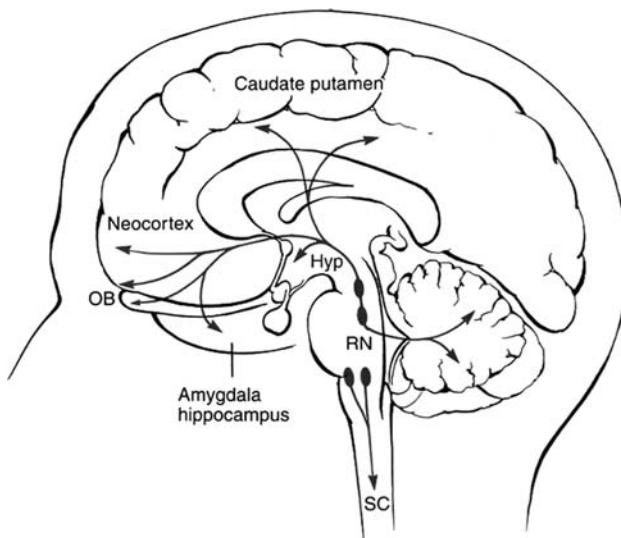


Fig. 13. The major serotonergic pathways in the brain originate in the midline pontine and upper brain-stem area known as the raphe nuclei (RN). The posterior cell groups project to the spinal cord (SC), and the anterior serotonergic axons parallel the noradrenergic nerves to innervate diffusely the cortex, striatum, hypothalamus (HYP), olfactory bulb (OB), amygdala, hippocampus, and cerebellum.

behavior. The serotonergic cells of the raphe possess a pacemaker-like activity that is modified by 5-HT autoreceptors and noradrenergic receptors. The rate of activity of these cells is high during wakefulness, low during sleep, and absent during *rapid eye movement* (REM) sleep. The raphe nucleus displays pacemaker-type activity similar to the sinoatrial node of the heart, which regulates the rate of the heart. Perhaps the raphe serotonergic neurons regulate the “rate of the brain function,” in a manner analogous to altering the clock speed of a computer CPU. Although the serotonergic system has been linked to and modulates such phenomena as sleep, vigilance, arousal, sensory perception, and emotion, it does not appear to generate these phenomena.

Pharmacologic observations have implicated the serotonergic system in higher cognitive function, schizophrenia, and hallucinations. Many of the hallucinogenic drugs resemble serotonin and interact with the 5-HT receptors. Dimethyltryptamine (DMT) and diethyltryptamine are hallucinogenic drugs of abuse, differing from serotonin by the addition of two methyl or ethyl groups on the amine terminal. The hallucinogenic drug lysergic acid diethylamide (LSD) also is chemically similar to serotonin and has been shown to interfere with autoreceptor function at the raphe nucleus and increase serotonergic firing rates.

Schizophrenia was once believed to be caused by the pathologic production of an abnormal serotonin, such as DMT.

7.3.4. AMINERGIC THEORY OF AFFECTIVE DISORDERS

The *affective disorders* are a group of psychiatric diseases that include mania and unipolar and bipolar depression. Bipolar depression is characterized by exaggerated swings in mood that may cycle over a period of months or years. It is a serious, debilitating disorder that can lead to suicide. About 80% of these patients respond to medication.

Although the medications fall into different groups, they all tend to make more biogenic amines available for receptor stimulation. The two major groups are the *tricyclic antidepressants*, which inhibit the uptake of the biogenic amines, and the MAO inhibitors, which inhibit their degradation. This information has prompted the *amine theory of affective disorders*.

There are still many unanswered questions regarding this theory. The specific amine that is responsible for the depressed mood is unknown. The tricyclic agents inhibit the uptake of norepinephrine, epinephrine, dopamine, and serotonin, and MAO is responsible for degrading all of the amine transmitters. There are selective uptake inhibitors for serotonin and norepinephrine, which are both effective in treating depression. The SSRIs are widely prescribed drugs and have been shown to be effective. All of the antidepressants take several weeks to alleviate depression, although they are immediately effective in inhibiting amine uptake or MAO (*in vivo* and *in vitro*). One explanation for the delay is that they lead to slow changes in receptor populations that result in stabilization of mood. Depression is still an ill-defined disease that may result from several biochemical imbalances.

7.4. Histamine

Histamine is found in mast cells and central neurons. This imidazole-containing substance has been studied since the early 1900s, when Henry Dale described its many actions. It was isolated and purified around 1930, and it has often been considered as a neurotransmitter candidate. Acceptance of this role for histamine, however, has been hampered by several factors. It is found in high concentrations in mast cells (also present in the brain), where it is important for immune responses. The concentration of histamine in the brain is about 50 ng/g, some of which is undoubtedly in mast cells. Administration of mast

cell-degranulating agents (e.g., histamine-depleting agents) produces a 50% reduction of brain concentrations of histamine. During postnatal development, when the blood-brain barrier matures, the elevated histamine levels and the number of mast cells concurrently decrease.

The histochemical techniques to visualize histamine-containing neurons were difficult to develop and are only now being applied. Because the enzyme responsible for synthesizing this compound, histidine decarboxylase, is very much like the amino acid decarboxylase that produces catecholamines and serotonin, the specificity of its localization is questionable.

7.4.1. HISTAMINE SYNTHESIS AND METABOLISM

Histamine is produced by the decarboxylation of the essential amino acid histidine, as shown in Fig. 14. The enzyme that catalyzes this reaction resembles L-amino acid decarboxylase, although it has been difficult to isolate from adult mammalian tissue. A fetal liver histidine decarboxylase has been purified and is believed to be similar to the enzyme in central neurons. Like the other decarboxylases, it requires vitamin B (e.g., pyridoxal phosphate) and can be inhibited by drugs that interact with the same step in catecholamine and indolamine transmission. Similar to tryptophan hydroxylase, the affinity (K_m) for histidine decarboxylase is higher than the amount of substrate (e.g., histidine) available for decarboxylation. Because the enzyme is not usually saturated, dietary intake of this essential amino acid may affect the activity of this system. Histidine loading increases the amount of histamine in the brain.

The major catabolic pathway for the elimination of histamine in the mammal is through N-methylation to methylhistamine (Fig. 14) by the enzyme histamine methyltransferase. This enzyme uses S-adenosylmethionine as the methyl donor. Methyl-histamine can be further catabolized by MAO.

7.4.2. HISTAMINE PATHWAYS

Using antibodies against histidine decarboxylase and histamine itself, nerve pathways have been observed that originate in posterior basal hypothalamus and premammillary areas. These histaminergic neurons appear to ascend through the medial forebrain bundle to innervate the forebrain, including cortical, thalamic, and limbic structures. Lesioning of the medial forebrain bundle causes a complete depletion of the norepinephrine and serotonin, which has a time course similar to the degeneration of axons after lesioning. Studies have shown that

Histamine Biosynthesis

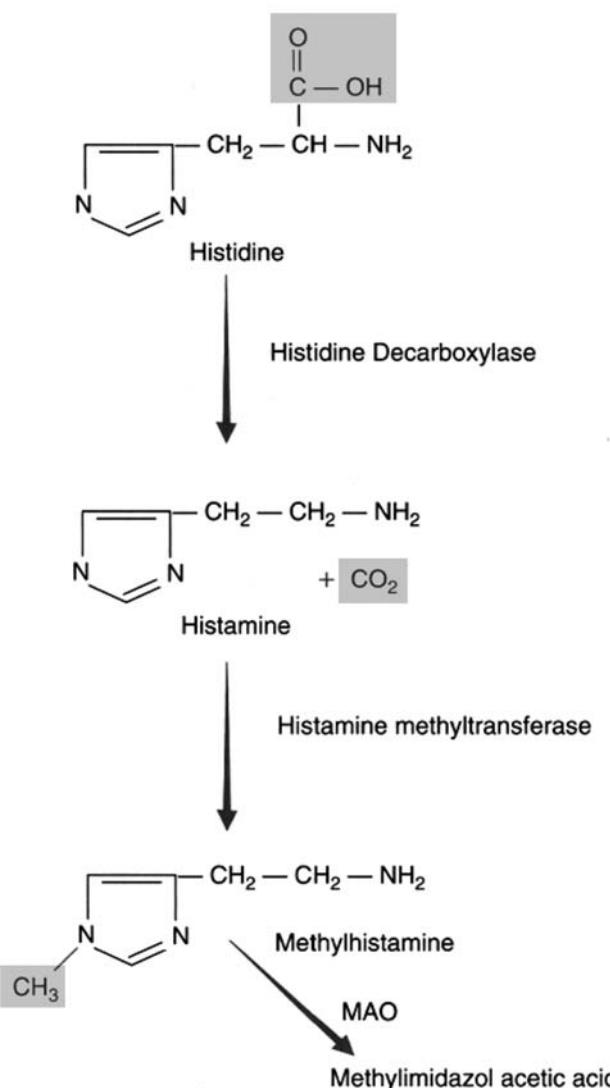


Fig. 14. Histamine biosynthesis and degradation. The amino acid histidine is decarboxylated by histidine decarboxylase to produce the neurotransmitter histamine and CO₂. Histamine is degraded by methylation initially to methylhistamine before being oxidized by monoamine oxidase (MAO) to methylimidazol acetic acid. The shaded groups indicate enzymatic additions or deletions.

similar lesions also cause a 70% decrease in histidine decarboxylase levels in the forebrain.

Many studies have indicated that central histamine pathways may be involved in the central control of autonomic and endocrine activity, food and water intake, and temperature regulation. Because blockers of the H₁ histamine receptor causes sedation, a role in arousal—like those of catecholamines and serotonin—is also suggested. This is easily

demonstrated in the allergic patient, in whom anti-histamines have the side effects of sleepiness and increased appetite.

This chapter does not cover all of the substances currently considered to be neurotransmitters, but rather focuses on those that are best-known, understood, and studied. Among the other potential candidates are taurine, octopamine, and ATP. The field offers a tremendous potential for further investigation of these candidates.

8. AMINO ACID NEUROTRANSMITTERS: GABA, GLUTAMATE, AND GLYCINE

Although the amino acid tyrosine is the common precursor for all three of the catecholamine neurotransmitters, glutamate is equally crucial for production of the amino acid transmitters in the CNS. Glutamate is a transmitter in the excitatory amino acid (EAA) pathway and is the precursor for the major inhibitory amino acid transmitter, GABA. The concentrations of these substances are almost 1000 times higher than those of the conventional amine transmitters in the brain, which may reflect their relative importance.

The EAAs, glutamate and aspartate, depolarize their postsynaptic target neurons and can be compared with the accelerator pedal of an automobile. GABA in the brain and glycine in the spinal chord hyperpolarize their target neurons and can similarly be compared with the brake pedal of the automobile. The inhibitory importance of GABA and glycine can be easily demonstrated if their receptors are blocked by drugs such as picrotoxin or strychnine, which block the GABA and glycine receptors, respectively. Administration of these drugs immediately induces life-threatening seizures and death.

Why were such important transmitter systems overlooked for so many years? The reason lies in the fact that amino acids are a common constituent of all cells. Although norepinephrine or serotonin could be specifically localized to certain neurons and brain areas, glutamate and glycine are universally present. New neurochemical, molecular, and immunochemical techniques have greatly expanded our understanding of the distribution and functioning of these transmitters.

8.1. GABA: Function and Distribution

GABA serves as the principal transmitter involved in internal circuits within specific brain areas. GABA is found in the brain, spinal cord, and retina, with

only trace quantities observed in other types of tissues or peripheral nerves. Quantitatively, the amount of the biogenic amine norepinephrine is in the range of nmol/g, but GABA can be measured within the brain in units of $\mu\text{mol}/\text{g}$. If the concentration of a transmitter is 1000-fold more, is it proportionally that much more important? The answer to this question is being sought in many laboratories.

8.1.1. GABA SYNTHESIS

The decarboxylation of glutamate to GABA is not very different from the decarboxylation of L-DOPA or tryptophan to dopamine and serotonin. The enzyme that does this is called glutamic acid decarboxylase (GAD), and it removes the α -carboxyl group to produce a γ -carboxyl amino acid. The biosynthesis and catabolism of GABA is shown in Fig. 15. Like the three other decarboxylases discussed in this chapter, GAD requires the cofactor pyridoxal phosphate (vitamin B₆). The saturation of GAD with its cofactor may be one of the rate-limiting steps in GABA synthesis, controlled by steric inhibition with ATP. This could explain why the concentration of GABA in the brain increases rapidly from 30% to 45% after death when ATP levels drop. The distribution of GAD does appear to be selective to endogenous GABA neurons, which generally have short axons. GAD is not present in glia or other types of neurons.

The GABA shunt, which is studied in conjunction with the Krebs cycle in biochemistry, is a unique feature of the brain and a viable form of energy production. The key aspects of this pathway are emphasized in Fig. 15. GABA is produced from α -oxoglutarate, through glutamate, degraded by two enzymes present throughout the brain, and reenters the Krebs cycle as succinic acid.

What is the significance of this energy-producing shunt? First, it generates ATP (three), although a little less efficiently (by one GTP) than the Krebs cycle. Estimates have indicated that the GABA shunt contributes to 10% to 40% of brain metabolism. Second, glial cells, which have high-affinity GABA pumps, are able to scavenge GABA and use it for energy. Third, glutamate is regenerated through the degradation of GABA in GABAergic nerves. Enzyme localization studies have shown that GAD is high in GABA-containing areas (e.g., GABA neurons), but the GABA-degrading enzymes, GABA transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH), are localized throughout the brain.

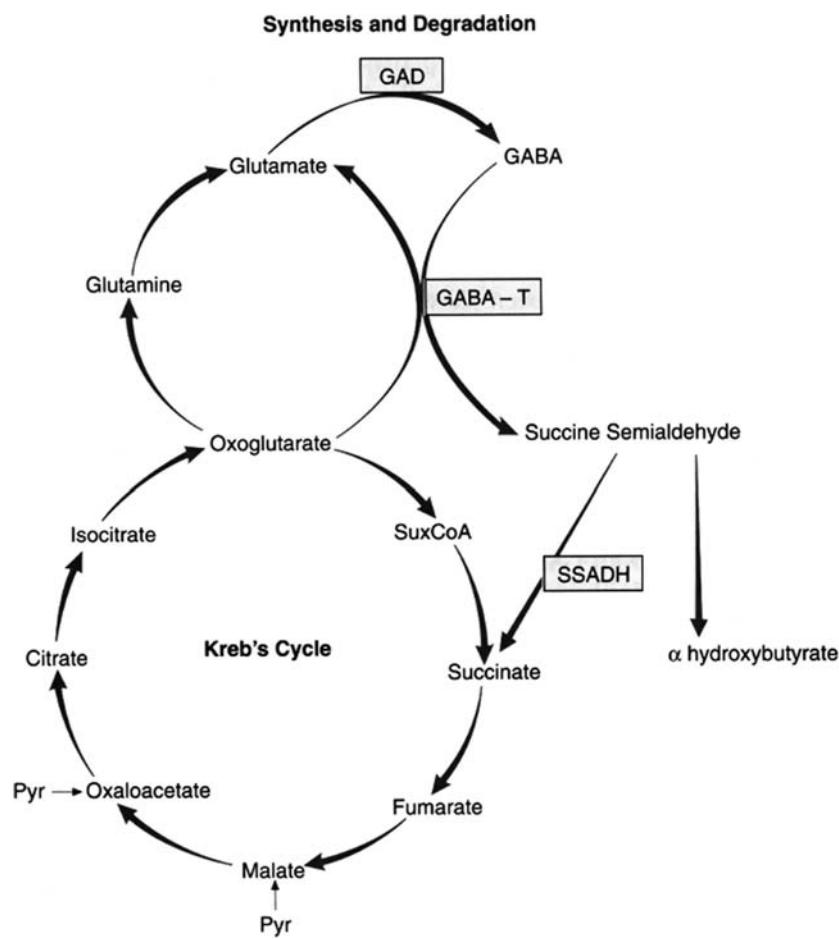


Fig. 15. γ -Aminobutyric acid (GABA) and glutamate synthesis and degradation are shown in relation to the Krebs cycle and GABA shunt. The important enzymes in the pathway are shown in the shaded boxes. GABA is synthesized by the enzyme glutamic acid decarboxylase (GAD) from glutamate. GABA can be degraded by GABA transaminase (GABA-T) to succinic semialdehyde in a reaction that regenerates glutamate from oxoglutarate. Succinic semialdehyde can reenter the Krebs cycle by the action of succinic semialdehyde dehydrogenase (SSADH) or can be degraded to α -hydroxybutyrate. The entire system is fueled by glucose, which enters the cycle after being converted to pyruvate (pyr) as indicated.

8.1.2. GABA METABOLISM

GABAergic transmission is terminated by uptake into neurons and glia, where it is metabolized by enzymes that can regenerate glutamate and produce more ATP. The GABA-uptake transporter has been characterized and cloned. It is a large protein that appears to have 12 membrane-spanning domains containing hydrophobic amino acids that can insert through the lipid bilayer of the membrane. Like the catecholamine transporter, it is Na^+ -dependent and has been shown to transport two Na^+ ions out of the cell in exchange for one GABA and one Cl^- ion. Because of the ionic redistribution, GABA uptake is electrogenic (e.g., changes the membrane potential) and can sequester GABA against a gradient of 10,000 to 1.

GABA is catabolized by several enzymes that are localized throughout the brain. GABA transaminase requires pyridoxal phosphate and may under certain circumstances compete with GAD for the cofactor. The K_m of GABA-T for vitamin B₆ is much lower than that of GAD. The overall effect of vitamin B₆ deficiency is an increased susceptibility to seizures related to decreased GABA synthesis. These seizures are rapidly reversed after pyridoxine is administered. Transamination of GABA to succinic semialdehyde by GABA-T results in the regeneration of glutamate for decarboxylation to GABA. GABA-T catalyzes the formation of succinic semialdehyde, which is metabolized by SSADH to succinate. The K_m of SSADH is so low (10^{-6} M) that very little of its substrate, succinic semialdehyde, ever accumulates in the

brain. An alternate pathway results in the formation of the metabolite γ -hydroxybutyrate.

8.1.3. GABA PATHWAYS

CNS localization studies have hinged largely on the immunocytochemical distribution of GAD. Many of these pathways are outlined in the brain map in Fig. 16. Mapping studies have shown that GABAergic pathways make up the major endogenous circuits within specific brain areas. Most of these have short axonal pathways that interact locally. The best-understood systems are those in the Purkinje cells of the cerebellar cortex that inhibit the deep nuclei and the GABAergic system in the striatum that projects to the substantia nigra and globus pallidus (GPe). Other systems that are believed to be GABAergic are the granule cells of the olfactory bulb, some of the amacrine cells of the retina, basket cells of the hippocampus, and a long ascending projection from the hypothalamus to the cerebral cortex (4).

The major function of the GABA system is inhibition of the target pathways. This is accomplished by the opening of chloride channels and the resultant hyperpolarization. Several drugs that interact with the GABA system illustrate the inhibitory function

of these nerves. GABA-blocking drugs such as picrotoxin or drugs that decrease the availability of GABA lead to convulsions similar to epileptic seizures. This transmitter system is believed to be important in the cause of epilepsy. Drugs that increase the amount of GABA or potentiate the action of GABA result in central inhibition and can lead to coma and death. Many drugs potentiate the GABA system, including the barbiturates, benzodiazepines (e.g., diazepam (Valium) and chlordiazepoxide (Librium)), anesthetic steroids, and alcohol. The antianxiety and sedative characteristics of these agents provide a good idea of some of the actions and functions of central GABA systems. It is possible to speculate that death is not such an unpleasant experience because of the massive disinhibition and increase in GABA activity found postmortem.

This is an interesting, seemingly well-designed, inhibitory transmitter system that generates metabolic energy when it is active, regenerates its own substrate, and limits the rate of its own activity through production of ATP. Thus, it is well-suited to be the major inhibitory transmitter system in the brain.

8.2. Glycine

Glycine is an inhibitory transmitter that is principally localized in the brain stem and spinal cord. The transmitter role of glycine, like glutamate, was disputed for years based on the fact that it is an amino acid found in all cells as an important constituent of proteins, peptides, and precursor for porphyrins and nucleic acids. The concentration of glycine is very high in the spinal cord, as is that of glutamate and of glutamine. Glycine is the simplest amino acid in the body and is not considered to be essential for the diet.

Although glycine could originate from many metabolic sources, it appears to be predominately generated from serine. This has been determined by following the incorporation of the radioactive carbon into glycine after administering various radiolabeled precursors. Similarly, although there are many possible degradation pathways, active uptake seems to be the preferred route of removal of this inhibitory amino acid from the synapse. Like the GABA and biogenic amine transporters, the glycine uptake mechanism is Na^+ -dependent. Radiolabeled glycine preloaded into neurons can be released from spinal cord slices by depolarization in a Ca^{2+} -dependent manner. Glycinergic neurons are believed to mediate motor and sensory functions in the spinal cord, brain stem, and the retina.

Like GABA, glycine has important anticonvulsant properties. However, the antianxiety and sedative

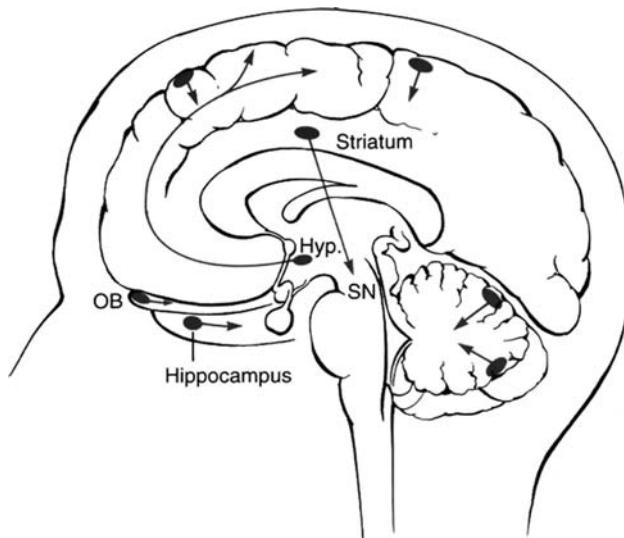


Fig. 16. The GABA-producing neurons in the brain consist mostly of interneurons with short axons that remain within the brain area from which the cells originate. GABA concentrations are about 100-fold higher than those of the catecholamines in most brain structures, including the cortex, limbic system, and cerebellum. In contrast, a group of GABA neurons in the striatum with long axons projects to the substantia nigra (SN), and another tract in the hypothalamus (Hyp) is believed to project to the forebrain. Tracts in the olfactory bulb (OB) and hippocampus are also depicted.

actions of glycine have not been demonstrated pharmacologically. The Renshaw cell of the ventral root is an interneuron that has been characterized in terms of its ability to alter alpha motor neuron activity. This glycinergic interneuron receives its input from a collateral axon of the alpha motor neuron and is stimulated by the release of acetylcholine when the motor neuron fires. The stimulated Renshaw cell then releases glycine onto dendrites or the cell body of the motor neuron to inhibit further motor activity. When this classic feedback mechanism is blocked by strychnine, the motor neuron fires without control, and convulsions result. Strychnine is a specific glycine-receptor blocker. It is a complex plant alkaloid found in the herb *Nux vomica*. It has been used as a rat poison and is an ingredient in herbal, homeopathic, and proprietary drugs, although there is no generally accepted rationale for its use. Sensory actions of glycine in the spinal cord are also suggested by the use of small amounts of an extract of *Nux vomica* to increase tactile sensations.

Glycine also appears to be an important component of glutamatergic transmission. One of the glutamate receptors (e.g., NMDA receptor) has a glycine-binding site that, when occupied, increases the frequency of Ca^{2+} -channel opening. This neuromodulatory response to glycine is not blocked by strychnine.

8.3. Glutamate

Glutamate is the major excitatory transmitter in the brain. Amino acids such as glutamate and aspartate stimulate (e.g., depolarize) many different types of neurons. For years, this action was considered to be nonspecific, which seemed to disqualify glutamate as a transmitter candidate. Glutamate and its precursor glutamine are found in very high concentrations in the brain and spinal cord. These high levels are apparently achieved without uptake from the periphery, as the influx of glutamate into the brain from the blood seems to be slower than its efflux from the brain to the periphery. The massive amounts of glutamate and glutamine in the brain arise from the metabolism of glucose by means of the Krebs cycle. Like glycine, these amino acids occur in all brain cells, including glia, which made it difficult for many years to map precise glutamate and aspartate pathways throughout the CNS. Now that several glutamate receptors have been identified and cloned, receptor subtype-specific antibodies have been generated and used to characterize the distribution of these receptors throughout the CNS.

Two possible pathways for the synthesis of glutamate in the CNS were discussed in the previous section on GABA and are diagrammed in Fig. 15. Synthesis can proceed from glucose via α -oxoglutarate (α -ketoglutarate) by the actions of GABA-oxoglutarate transaminase (GABA-T) or from glutamine by means of glutaminase. The synaptic activity of glutamate is terminated through the activation of high-affinity, Na^+ -dependent glutamate transporters that sequester glutamate inside neurons and glia. In some brain areas, the glial uptake pathway is particularly important. Glia convert glutamate to glutamine via the enzyme glutamine synthetase and then export it for surrounding neurons to take up via a low-affinity uptake system. In the dentate gyrus, for example, the active uptake of glutamine by neurons is required for continued availability of glutamate for signal transmission. The system has many similarities to the acetylcholine system, in which uptake of a precursor is a rate-limiting consideration in transmission.

EAA nerves serve the function of the major projection neurons between brain areas. Essentially every outgoing (e.g., efferent) system of the cortex appears to use glutamate, including the corticostriatal, thalamic, bulbar, and pontine pathways. The mossy fiber afferents of the cerebellum and pathways in the olfactory lobe may also use glutamate. Some of the glutamatergic pathways in the CNS are diagrammed in Fig. 17.

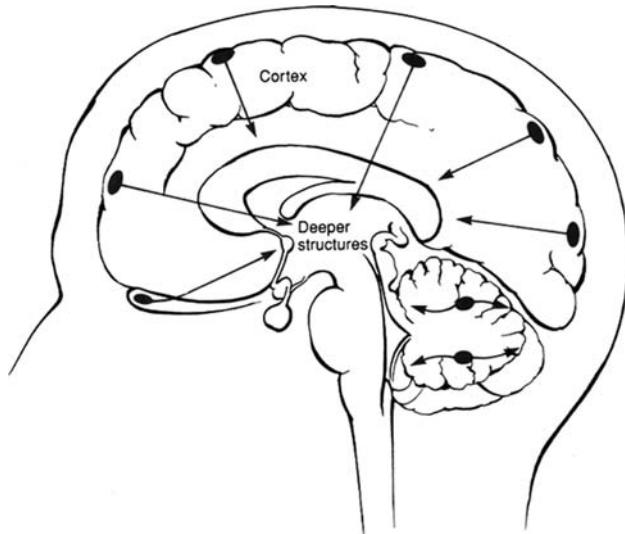


Fig. 17. Central excitatory amino acid (EAA) or glutamate pathways are the major efferent pathways from one brain area to another. These neurons possess long axons that project from the cortical and cerebellar areas to almost all brain areas. Concentrations of this transmitter are 1000-fold higher than those of the catecholamines. The glutamate neuronal pathways are denoted by black ovals and arrows.

As can be seen from the distribution of fibers, the glutamatergic pathways appear to be the major stimulatory or driving pathway within the CNS. Pharmacologically, the effects of underactivity of this system are unknown, although many of the antagonists such as ketamine and phencyclidine (both hallucinogenic) have anesthetic properties. Overactivity of the glutamate system and the action of some highly potent agonists such as quisqualic acid, ibotenic acid, and kainic acid can lead to overstimulation and neurotoxicity.

GABA and glutamate appear to be the major inhibitory and excitatory transmitter systems in the CNS and provide braking and acceleration systems, respectively, for neural activity. It is fascinating that these major systems use substances that are so intimately involved in brain metabolism. Through these systems, central energy metabolism, transmission, and nerve activity are inseparably linked.

9. ENDOCANNABINOIDS

Although the neuroactive properties of the *Cannabis* plant have been appreciated for many centuries, it is only in the past 15 years that investigators have begun to understand and characterize the neurochemical system through which the active component of cannabis, Δ^9 -tetrahydrocannabinol, produces its effects, and the nature of the endogenous ligand. The structure of the endogenous cannabinoid unexpectedly turned out to be an arachidonic acid derivative, arachidonoyl ethanolamide (AEA), and was dubbed “anandamide” after the Sanskrit *ananda*, which means “inner bliss” (5, 6). Subsequently, 2-arachidonoylglycerol (2-AG) has been shown to be the other major endocannabinoid produced in the brain. Unlike other transmitters, endocannabinoids are not stored preformed for release, but are produced “on demand” as needed; in this respect only, they resemble nitric oxide (*vide infra*).

The major synthetic pathway for AEA begins with the *N*-acyl transferase-mediated addition of an arachidonate group from another phospholipid to the amino head group of phosphatidylethanolamine (PE) in the plasma membrane. This intermediate compound is then cleaved by *N*-acyl PE phospholipase D (PLD) into AEA and phosphatidic acid. One isoform of *N*-acyl PE PLD is Ca^{2+} dependent, providing a means for regulating AEA production that is sensitive to both pathologic and physiologic signals. 2-AG is synthesized from phosphatidylinositol by the

action of phospholipase C (PLC) and diacylglycerol lipase or phospholipase A₁ and lyso-PLC.

The physiologic response to endocannabinoids is mediated largely by two G protein-coupled receptors, CB₁ and CB₂. Whereas CB₁ receptors are found throughout the body, predominately in neurons of the central and peripheral nervous systems, CB₂ receptors are found on activated microglia and immune cells and are not expressed in neurons. The CB₁ receptors in the CNS are usually found on GABAergic interneurons that also express CCK. In the cortex, areas that are richest in CB₁ receptors include the neocortex, basolateral amygdala, cortical amygdaloid nuclei, anterior cortical nucleus, and the hippocampus. In the mid-brain, CB₁ receptors are largely associated with cholinergic neurons in the lateral and medial septum, the vertical and horizontal diagonal bands, and basal nucleus of Meynert. Other areas that are rich in CB₁ receptors include the cerebellum, the globus pallidus, and the substantia nigra.

Coupling primarily to G_{i/o} proteins, the main biological responses produced by these receptors are the inhibition of adenylyl cyclase, inhibition of voltage-activated Ca^{2+} channels, and the activation of K⁺ channels. The observable physiologic effects of activating these receptors vary with the individual but include euphoria, muscle relaxation, hypothermia, and reflex tachycardia. Stimulating these receptors also produces effects that have potential clinical applicability including analgesia for neuropathic pain, appetite stimulation, and antiemetic action.

The termination of endocannabinoid signaling is accomplished by a transport mechanism that meets four key criteria for a carrier-mediated process: fast rate, saturability, substrate selectivity, and temperature dependence. Once sequestered inside a cell, AEA and 2-AG are rapidly hydrolyzed by, respectively, fatty acid amide hydrolase (FAAH) or monacylglycerol lipase. The distribution of this transport mechanism appears to be largely congruent with the observed physiologic effects: the motor, somatosensory, and limbic areas of the cortex and the striatum contain the highest levels, and the amygdala, hippocampus, hypothalamus, septum, substantia nigra, and thalamus all exhibit detectable transport.

Perhaps the most intriguing discovery regarding the endocannabinoids is their role in mediating two forms of retrograde transsynaptic transmission, depolarization-induced suppression of inhibition (DSI), and depolarization-induced suppression of excitation (DSE). These phenomena have been most

clearly demonstrated in the hippocampus and the cerebellum, respectively, and provide additional mechanistic information that may help explain some of the behavioral effects of cannabinoids as they relate to memory and depressed motor function. Thus, cannabinoids appear to play an important role in modulating synaptic plasticity, timing related to phase-locked signaling, and oscillatory circuits associated with higher cognitive functions.

10. NITRIC OXIDE

In the late 1970s, several groups demonstrated that nitroso and related nitro compounds, believed to decompose or be converted into nitric oxide (NO), were capable of activating the cytosolic form of guanylate cyclase to stimulate cyclic GMP production in mammalian tissues. These observations led others to consider that the well-known vasodilating effects of nitro compounds, such as *nitroprusside* and *nitroglycerin*, might be caused by the conversion of these compounds to NO by smooth muscle. Subsequent studies did show that NO is a potent smooth-muscle relaxant and that the resulting vasodilation is produced by the actions of cyclic GMP. Together, these observations suggested that smooth muscle and possibly other tissues possessed the capacity of generating NO from an unknown donor compound. The mystery was finally solved with the demonstration that L-arginine is converted to L-citrulline and NO by NO synthase in an NADPH-dependent manner.

Although most of the early NO research efforts were focused on vascular smooth muscle and platelets, the activation of guanylate cyclase from neuronal cells by L-arginine was demonstrated well before the NO synthetic pathway had been worked out. Eventually, the existence of the NO synthetic pathway in brain tissue was demonstrated, and the enzyme itself was purified from rat cerebellum. As other isoforms were discovered and characterized, the brain-derived isoform was designated neuronal NO synthase (nNOS). It is constitutively expressed in many different types of neurons and is particularly abundant in the molecular layer of the cerebellum and the pediculopontine tegmental nucleus of the brain stem. Although a specific function for NO in the CNS has not been determined, nNOS has been shown to colocalize with NMDA receptors, and glutamate activation of these receptors is associated with increased production of NO. Because NMDA-receptor activation has been implicated in long-term potentiation (LTP) in the hippocampus and with

neurotoxicity, NO production may also be involved in mediating these phenomena (7).

In the peripheral nervous system, both vascular and nonvascular smooth-muscle relaxation appear to be mediated by NO functioning as a neurotransmitter. The myenteric plexus of the gastrointestinal tract contains neurons that express nNOS and inhibit peristalsis when stimulated. NO functions as a neurotransmitter in the erectile tissue of the penis, where it mediates penile erection by inducing relaxation of both vascular and nonvascular smooth muscle. Recognition of this response pathway led to the development of the drug sildenafil (Viagra) for the treatment of male impotence. This drug inhibits selectively the isoform of phosphodiesterase (PDE5) expressed in this tissue that metabolizes the cyclic GMP produced in response to NO.

11. NEUROACTIVE PEPTIDES

A variety of neuroactive peptides found in the brain are synthesized in the neuronal-cell body and transported axonally to the nerve terminal. Peptidergic transmission in the CNS is different from that of the small-molecular-weight transmitters. More than 50 peptide transmitters have been described in the CNS, and more are being discovered every year. This may not be so surprising, considering the fact that the human brain contains approximately 10^{12} neurons.

The history of neuropeptides can be broken down into two periods: a 40-year period from about 1930 to 1970, when only a few peptides were chemically characterized, and the 37-year period from 1970 to the present, during which many neurally active peptides have been discovered and sequenced, mostly because of newer techniques and advances in molecular biology.

The first peptide transmitter to be identified, *substance P*, was discovered serendipitously around 1930, when U.S. Von Euler and John Gaddum were screening various tissues for concentrations of acetylcholine. A substance was found in the gut and in the brain that lowered blood pressure and increased gastrointestinal activity. The substance was not acetylcholine and was called substance P because it was a powder that resembled a protein in several ways. The neurohypophyseal peptides, *oxytocin (OT)* and *vasopressin*, were isolated and characterized in the 1950s, partially because of the immense concentrations that were stored in the neural lobe of the pituitary. In a common laboratory animal, the white rat, almost half

of a microgram of each of these peptides is stored and released into peripheral blood to effect physiologic functions such as water retention and lactation. Because of the very small size of the neural lobe and the amount of peptide present, it was relatively easy to extract and characterize these peptides, each of which is nine amino acids long. The structure of substance P was determined by amino acid sequencing around 1970, when this new method became available. At that time, myriad new brain peptides were isolated and characterized.

11.1. Neuropeptides Are Synthesized in the Cell Body

The brain peptides are present in the axon in large secretory granules that are synthesized in the cell body and are moved to the nerve terminal by axoplasmic transport. The actions of these peptides on their target cells take some time to occur because their receptors are of the G protein-coupled variety. Their rate of synthesis is also slow when compared with that of the catecholaminergic and amino acid transmitters. The process of *peptide synthesis* begins with the nuclear translation of specific messenger RNAs and their binding to ribosomes. When mRNA is translated into protein, a 20- to 30-amino-acid sequence known as the signal peptide is first formed. This hydrophobic sequence inserts itself into the endoplasmic reticulum (ER) and is followed by the gradually elongating protein chain. Some peptide processing occurs in the ER. The protein may be glycosylated with a mannose-containing sugar that is attached to asparagine residues (flanked by other specific amino acids). These specific sequences of amino acids are called *consensus sequences*. In the ER, the protein is folded, and disulfide bonds are formed. The disulfide isomerase that accomplishes this is not entirely characterized, but these bonds are important for maintaining the folded structure of the neuropeptide.

The mature propeptide is transported to the Golgi apparatus, where it can be further processed, sorted, and packaged into secretory granules. Much of the processing of the precursor to the active neuropeptide occurs within the secretory granule. These granules have a membrane-bound proton pump that maintains an acidic pH. An example of neuropeptide processing, using oxytocin, is shown in Fig. 18. Peptides are represented (by convention) with the amino-terminal on the left and carboxyl-terminal on the right.

Enzymes belonging to the subtilisin family of serine proteases are responsible for cleaving prohormones at paired basic amino acid residues (lysine and arginine). These enzymes have the characteristic active-site “catalytic triad” (D,H,S) and are active at an acidic pH (4.0 to 5.0) that allows them to work within the secretory granule. In the case of OT, this produces two products: an extended OT containing Gly-Lys-Arg on the carboxyl-terminal end and a 93-amino-acid protein called neuropephsin. Some proneuropeptides have more than a single neuropeptide sequence within their precursor. For example, proenkephalin-A has seven enkephalin sequences in the single precursor, and hypothalamic thyrotropin-releasing hormone (TRH) has five of these tripeptide sequences. The proteases that process the prohormone may differ in specificity between various tissues. For example, in the anterior pituitary, the hormone precursor, pro-opio-melanocortin (POMC), is converted into corticotropin (ACTH) and the opiate peptide β -endorphin. In the intermediate lobe, this same precursor POMC is cleaved to melanocyte-stimulating hormone (MSH), a peptide called corticotropin-like intermediate-lobe (CLIP), and β -endorphin.

The next enzymatic step eliminates the basic amino acids from the carboxyl-terminal of the neuropeptide. This enzyme is carboxypeptidase E/H, which has an acidic pH maximum and (in the case of OT) yields the nonapeptide with an additional glycine. Amidation is the next step in peptide synthesis, accomplished by an enzyme known as peptidylglycine α -amidating monooxygenase (PAM). Many peptides are amidated on the carboxyl-terminal end, including TRH, gonadotropin-releasing hormone, OT, vasopressin, angiotensin II (AII), substance P, and vasoactive intestinal peptide (VIP). The enzyme cleaves the additional glycine (of extended oxytocin or TRH) at the α -carbon atom, leaving the amide attached to the carboxyl residue (Fig. 18). In terms of cofactor requirements, this enzyme is similar to dopamine- β -hydroxylase. As is the case for norepinephrine biosynthesis, copper and reduced ascorbic acid are required for the amidation of peptides to occur within the vesicles.

Much of this peptide processing occurs as the secretory granule is being transported down the axon to the nerve terminal. An investigator can follow the maturation of some peptides from the cell body down the length of the axon to the nerve terminal. Peptide-containing granules are usually larger

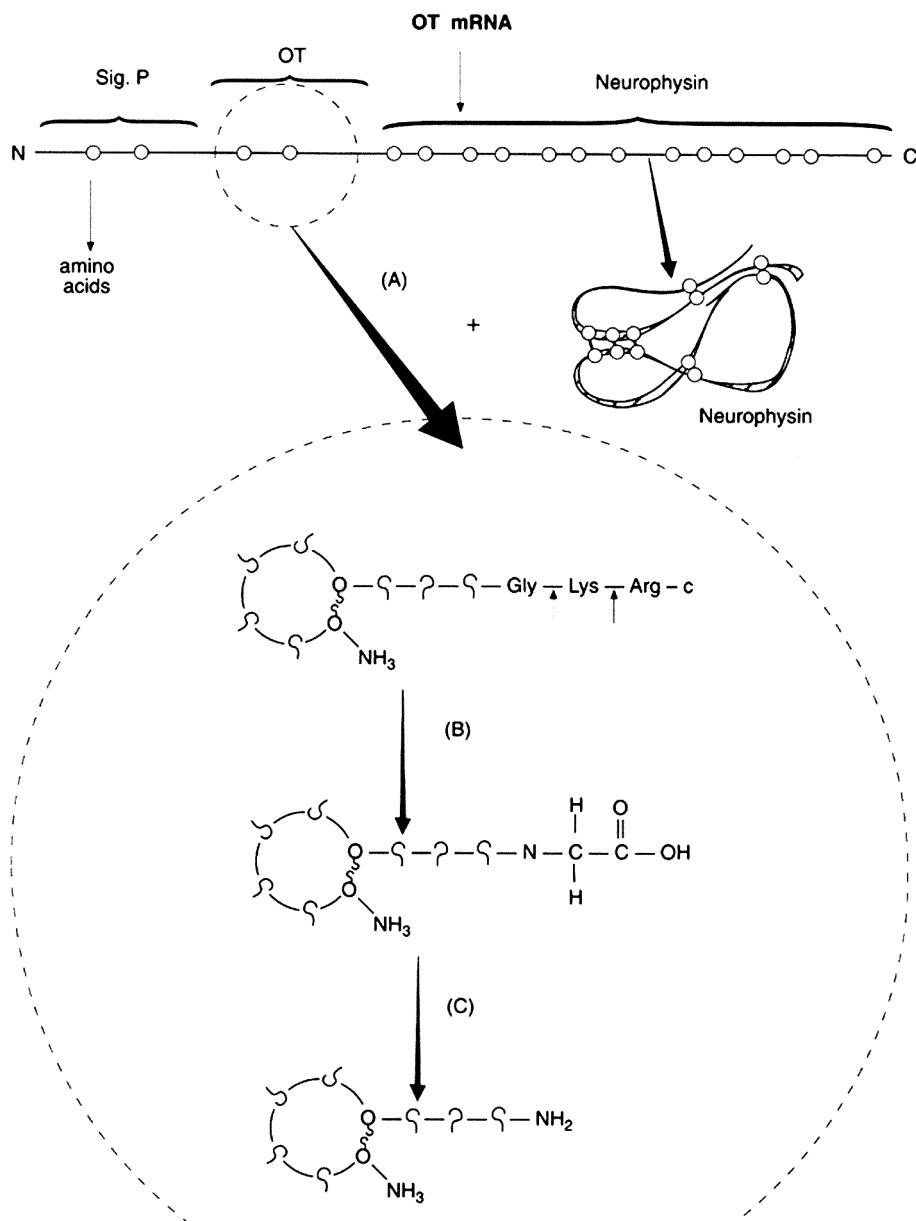


Fig. 18. Peptide biosynthesis in the CNS. Neuropeptides in the CNS are synthesized on ribosomes in the cell bodies of peptidergic neurons. This diagram describes the synthesis of oxytocin in the hypothalamus. The pro-neuropeptide contains the natural neuropeptide (OT) flanked by a signal peptide (Sig. P) and a binding protein known as neurophysin. The shaded dots on the propeptide represent the 18 cysteine residues (drawn relatively to scale). In the ER and Golgi apparatus, disulfide bonds are formed and the peptide is packaged in secretory granules. Within the secretory granule, the propeptide is cleaved by a serine protease (A) at a pair of basic amino acids. The extended OT is then acted on by a carboxypeptidase (B) to remove the extended basic amino acids and peptidyl glycine α -amidating monooxygenase (C) to produce the biologically active neuropeptide. The activity of these three enzymatic steps occurs in the synaptic vesicle while it is being transported to the axon terminal.

than fast transmitter-containing granules. When they exist as co-transmitters, they can be differentially released, depending on the frequency of nerve action potentials and distribution of Ca^{2+} channels. This has

been most completely worked out in the parasympathetic innervation of the salivary gland, where low-frequency stimulation releases acetylcholine and high-frequency stimulation causes the secretion of VIP.

11.2. Neuropeptide Metabolism

Termination of neuropeptide responses is achieved principally through enzymatic degradation. Uptake and reuse of peptides are not options, as there are no known peptide transporters. The enzymes that degrade peptides are called peptidases and are classified by the amino acids or metals that are involved in their catalytic site (e.g., serine and metalloproteases) or by the location of the cleavage site of the peptide. Endopeptidases cleave internal peptide bonds at specific sequences. An example is the enzyme that degrades the opiate peptides, sometimes called enkephalinase. Exopeptidases remove one or two amino acids from the carboxyl or amino terminus of the peptide. The carboxypeptidases that remove the basic amino acids from peptide precursors have already been discussed. Angiotensin II-converting enzyme (ACE), which removes a carboxyl-terminal dipeptide from its substrate, is the target for an important class of drugs used to lower blood pressure. Aminopeptidase A selectively removes acidic amino acids from the amino-terminal end of the peptide. A variety of peptidases is found in extracellular fluid or circulating in the blood, including aminopeptidase A and M, cathepsin D, and ACE.

Some of the characteristics of neuropeptides partially protect them from degradation by the peptidases. For example, the disulfide bonds and carboxyl-terminal amidation of OT and vasopressin are essential for biologic activity and protect these peptides from immediate destruction by circulating peptidases.

11.3. Families of Neuroactive Peptides

The peptides can be subdivided into several families of neurotransmitters. Because much of this information is discussed elsewhere in the text, only their major groupings, distribution, and some aspects of their function are discussed here.

11.3.1. NEUROHYPOPHYSIAL PEPTIDES

Hypothalamic neurons that secrete neurohypophysial peptides project to the posterior pituitary and higher brain centers. OT and vasopressin are synthesized in specific nuclei of the anterior hypothalamus and are transported by axons through the internal layer of the median eminence (ME) to the posterior pituitary (e.g., neural lobe). This small bundle of axon terminals and pituicytes stores large amounts of both peptides, which contributed to their being among the first neuropeptides to be isolated and characterized.

Vasopressin is secreted into the blood, where its hormonal functions include constriction of vessels, as its name implies, and inhibition of water loss by the kidney (hence the name antidiuretic hormone). OT is usually associated with reproductive function and is released to induce contraction of the uterus during parturition and the letdown of milk during suckling and lactation.

The axons of the neurons that produce these peptides project to other areas of the brain, brain stem, and spinal cord. Vasopressin has been associated with memory consolidation in the limbic system, and OT has been implicated in memory, maternal, social, and sexual behavior, and autonomic activity.

11.3.2. HYPOTHALAMIC NEUROPEPTIDES

The hypothalamus of the midbrain regulates autonomic functions, behavioral processes, and the endocrine system. Several small peptides reach the anterior pituitary through a portal blood system and regulate most humoral function. *Thyrotropin-releasing hormone* is a tripeptide that causes the release of thyroid-stimulating hormone (TSH) from the pituitary. *Gonadotropin-releasing hormone* is a decapeptide that releases pituitary luteinizing hormone (LH) and follicle-stimulating hormone (FSH) peripherally, and it has been implicated in some sexual behaviors, such as lordosis in the rodent.

Somatostatin (SS) occurs as both a tetradecapeptide (14 amino acids) that inhibits the release of growth hormone from the pituitary and as a 28-amino-acid form found primarily in the gut where it inhibits the secretion of acid and gastrin. It has also been found in many higher brain areas, including the cortex and limbic system. Present in peripheral nerves, SS also plays a role in sensory function.

Corticotropin-releasing hormone (CRH) is found in many higher brain areas. It is responsible for regulating the secretion of ACTH and modulating the immune system. CRH activates a G protein-coupled receptor (GPCR) that stimulates adenylate cyclase, increasing cAMP levels and activating protein kinase A.

11.3.3. OPIOID PEPTIDES

In the mid-1970s, a peptide-like substance was isolated from the brain that displaced morphine from a membrane-bound receptor present in many areas of the mammalian brain. Morphine, which is an opium derivative from the dried resin of the oriental poppy plant, has been used for centuries to alleviate pain and as a drug of abuse. The discovery of this brain

peptide answered a question that had been troubling pharmacologists and neurobiologists for some time—what is the endogenous substance that these plant alkaloids mimic so well?

Opioid peptides are found in almost all areas of the brain. Three major precursors give rise to the main opiate peptide groups: β -endorphin, the enkephalins, and the dynorphins. POMC, which was previously discussed, is the common precursor for ACTH and β -endorphin. It is present in the anterior and intermediate lobes of the pituitary and in an ascending group of neurons in the hypothalamus.

Opioid peptides have similar pentapeptide sequences. Proenkephalin-A gives rise to several pentapeptides (e.g., five methionine-enkephalins [Met-ENKs] and one leu-enkephalin [Leu-ENK]) that have potent opiate-like effects. Proenkephalin-B is the precursor for several leu-enkephalin pentapeptides and the dynorphins. The common feature of all of these opiate peptides is the pentapeptide sequence Tyr-Gly-Gly-Phe-Met (or Leu), which seems to be important for binding to the opiate receptors that have been described. This family of neuropeptides is important in analgesia and in a variety of other important inhibitory functions. The enkephalins and dynorphins are present throughout the CNS, particularly in the striatum, the limbic system, the raphe nuclei, and the hypothalamus.

11.3.4. BRAIN-GUT PEPTIDES

Since the discovery in 1930 of substance P, several peptides that are found in high concentrations in the gastrointestinal tract have been isolated and mapped in the brain. It has been proposed that the gastrointestinal tract has its own endogenous brain, known as the *enteric nervous system*, which is separate from the autonomic nervous system. The gut peptides that are also found in the brain include VIP, cholecystokinin (CCK), gastrin, secretin, neuropeptid Y (NPY), and many others. These peptides have been implicated in several physiologic processes, including pain, temperature regulation, satiety and hunger, and nausea and function as major co-transmitters in the autonomic nervous system (e.g., VIP and NPY).

11.3.5. OTHER NEUROPEPTIDES

The list of neuropeptides that have been isolated and characterized in the brain and periphery is still growing. *Angiotensin II* (*AngII*) and *bradykinin* are vasoactive peptides that have been localized within the brain. *AngII* has potent effects on water and fluid intake when injected directly into

the hypothalamus. *Orexin A and B* (*hypocretin-1 and -2*) are produced in the posterior lateral hypothalamus and are now recognized as important regulators of sleep/wakefulness. Abnormally low CSF orexin levels are highly correlated clinically in narcoleptic patients with cataplexy. *Calcitonin gene-related peptide* (CGRP) was accidentally discovered when the gene for calcitonin in the thyroid was sequenced and translated into protein. Antibodies against a peptide coded for by the gene reacted strongly with peptidergic pathways in the trigeminal nerve and hypothalamus. CGRP is a vasodilator, and recent studies have shown that CGRP receptor antagonists may be useful in treating migraine headaches. *Galanin* is another peptide that is widely distributed within the CNS, and it appears to be involved in regulating a variety of behaviors, including feeding, osmoregulation, nociception, learning and memory, anxiety, and the neuronal regenerative response to injury.

12. CONCLUSION

In this chapter, the basic neurochemical framework involved in the synthesis, degradation, and distribution of the majority of the known signaling molecules within the CNS has been summarized. It is highly likely that by the time this text is revised again, there will be newly discovered signaling molecules to be covered. Beyond the simple understanding of the mechanics of signal molecule production and termination, this knowledge is now being used to help understand the biochemical/genetic basis for various neurologic diseases through the discovery and functional characterization of protein polymorphisms in these synthetic and degradative enzymes. In turn, this genetic information can be used to “fine tune” pharmacotherapy for the treatment of these disorders. Finally, as we learn more about the mechanics of regulating individual signaling systems, we are better equipped to investigate and understand how these systems are interacting with each other to produce complex behaviors.

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Parkinson's Disease

Gregory Cooper, Gerald Eichhorn and Robert L. Rodnitzky

In 1817, the English physician James Parkinson published a monograph entitled *An Essay on the Shaking Palsy*. He described six persons afflicted by a condition characterized by a flexed posture, slowness of movement, a tendency to walk rapidly with small steps, and an associated tremor of one or more extremities. Not all of the six cases were Parkinson's personal patients. Some were observed on the streets of London. To Parkinson's description of tremor and slowness of movement, the modern neurologist would add rigidity and loss of postural reflexes to complete the typical symptom complex of Parkinson's disease. Although the exact etiology of sporadic Parkinson's disease remains unknown, it appears to ultimately result from degeneration of nigral neurons within the brain.

PARKINSON'S DISEASE SYMPTOMS

Parkinson's Tremor Is Most Prominent at Rest

Tremor is prominent in a variety of neurologic conditions, many of which are confused with Parkinson's disease. However, unlike most tremors, the parkinsonian tremor is most prominent at rest and is typically suppressed, at least temporarily, during action. In essential tremor, the condition most commonly confused with Parkinson's disease, tremor is typically absent at rest and only appears when the affected body part assumes a sustained, active posture. For instance, in essential tremor involving the hands, the tremor may only appear while holding the outstretched hands in front of the body or when attempting to bring a coffee cup to the mouth. The

distribution also helps to identify Parkinson's tremor. In Parkinson's disease, the tremor usually affects the extremities, especially the hands. It occasionally affects the mandible, but unlike essential tremor, it almost never affects the head.

The term *pill-rolling tremor* is often used in Parkinson's disease. It refers to the tendency of the thumb and index finger to approximate one another while trembling as though an object were being rolled between the two fingers. This term is a reference to the turn-of-the-century technique used by pharmacists to fashion a pill by rolling a soft substance between the thumb and index finger. Like many of the symptoms of Parkinson's disease, tremor is often asymmetric, and in the early stages of the illness, it may be exclusively on one side. The typical frequency of the parkinsonian tremor is 4 to 6 Hz.

Parkinsonian Rigidity Differs from the Rigidity of Upper Motor Neuron Lesions or Other Basal Ganglia Disorders

In Parkinson's disease, limb rigidity can be demonstrated throughout the entire range of a large-amplitude passive movement. In contrast, the stiffness associated with an upper motor neuron lesion such as a cerebral infarction is referred to as *clasp-knife rigidity*, because there is considerable resistance at the beginning of the examiner's passive movement, which suddenly gives away as the passive movement is continued. When the examiner attempts to demonstrate parkinsonian rigidity, a ratchet-like sensation known as *cogwheeling* can often be felt as the limb is moved. In Parkinson's disease, rigidity involves the extremities more than

the axial musculature. In other degenerative conditions that mimic Parkinson's disease, such as progressive supranuclear palsy, axial rigidity predominates.

Bradykinesia Is One of the Most Disabling Symptoms of Parkinson's Disease

Bradykinesia refers to a slowness of movement or inability to initiate movement. As a result of this dysfunction, automatic movements such as swinging the arms while walking become diminished or are totally lost. Similarly, eye blinking is decreased, and the normal range of facial expression is lost, resulting in a typical fixed stare. This diminution of facial expression is termed *hypomimia*. When severe slowness of movement evolves into a lack of movement, it is referred to as *akinesia*.

Patients with severe bradykinesia or akinesia find it difficult to perform motor tasks that require repetitive motions, such as finger tapping or combing the hair, and tasks that require manipulation of small objects, such as placing a button through a button-hole. Difficulty in initiating movement results in the inability to arise from a chair, exit a car, or roll over in bed. Of the four cardinal clinical features of Parkinson's disease (tremor, rigidity, bradykinesia, and loss of postural reflexes), bradykinesia is the most severely disabling.

Another common clinical manifestation of bradykinesia is inability to properly manipulate a pen or pencil while writing. The resultant handwriting, referred to as *micrographia*, classically becomes progressively smaller as the affected person writes, reflecting an inability to carry out sustained repetitive movements in a normal fashion.

Like many of the clinical signs of Parkinson's disease, the exact physiologic basis of bradykinesia is not completely understood. A loss of dopaminergic input into the striatum with subsequent disruption of motor subcircuits involving primary motor cortex may be involved. The phenomenon of kinesia paradoxia, wherein an ordinarily bradykinetic patient demonstrates an unexpectedly rapid motor response to a sudden stimulus such as a thrown ball, suggests that alternate motor pathways may exist in individuals who can support movements of normal range and speed.

Impairment of Postural Reflexes Is Typically a Late Development in Parkinson's Disease

Postural reflexes are tested by applying a backward-directed perturbation to both shoulders

in an attempt to pull a person off his base. The normal person takes one step at most to compensate for the perturbation. The Parkinson's patient with impaired postural reflexes cannot right himself and continues to move backwards with a series of small and uncontrollable steps until stopped by the examiner. This inability to stop oneself from propelling backwards is referred to as *retropulsion*. The tendency to take uncontrollable steps in increasingly rapid fashion in the forward direction is referred to as a *festination* or *propulsion*. Patients with moderate to severe impairment of postural reflexes are at great risk for falls, because they cannot recover from the slightest naturally occurring perturbation, such as tripping over the edge of a rug.

The Cardinal Symptoms of Parkinson's Disease Can Appear in Different Combinations

Not all patients have all four of the cardinal symptoms of Parkinson's disease. Isolated resting tremor is a common presenting symptom of Parkinson's disease, and in some patients, it remains the predominant symptom for the entire course of the patient's illness. In a small percentage of patients, tremor never develops, and the parkinsonian syndrome consists solely of rigidity and bradykinesia with or without loss of postural reflexes. The absence of resting tremor makes establishing a diagnosis of Parkinson's disease slightly more difficult. If bradykinesia appears in isolation, it is commonly manifested as a change in handwriting or difficulty in performing fine motor tasks.

AGE-RELATED INCIDENCE OF PARKINSON'S DISEASE

In the United States, the estimated prevalence of Parkinson's disease is approximately 187 per 100,000 persons in the general population, with an annual incidence of 20 per 100,000. Although occasionally beginning in persons as young as 30 years of age, it classically presents in the sixth and seventh decades. Epidemiologic surveys suggest that the prevalence per 100,000 is between 30 and 50 in the fifth decade and between 300 and 700 in the seventh decade. The increasing occurrence with age has led to the speculation that normal age-related abiotrophy of dopaminergic cells is a contributory factor in the etiopathogenesis of the illness.

Whereas the majority of cases of Parkinson's disease are thought to be sporadic, the potential importance of genetic factors is being increasingly

recognized. A number of kindreds have now been identified in which affected individuals demonstrate a Parkinson's-like phenotype. In most cases, this follows an autosomal dominant pattern and may involve mutations of the alpha-synuclein or tau genes. Mutations of the parkin gene can result in an autosomal recessively inherited syndrome. It is hoped that further identification and characterization of kindreds will lead to better understanding of the factors leading to sporadic Parkinson's disease. However, it is noted that the large majority of patients do not suffer from a clearly defined genetic syndrome. Supporting the relatively minor role of heredity in these cases is the fact that twin studies have not consistently suggested a contribution of genetic factors in young-onset Parkinson's disease. It has been proposed that the inconsistent results from genetic studies may result from a complex interaction between genetic and environmental factors. In other words, an individual may inherit a genetic risk or predisposition for Parkinson's disease but only develop this condition after a particular environmental exposure.

THE LEWY BODY IS THE PATHOLOGIC HALLMARK OF PARKINSON'S DISEASE

In Parkinson's disease, there is severe neuronal loss in the pars compacta of the substantia nigra. Many of the remaining neurons contain a Lewy body, an eosinophilic cytoplasmic inclusion surrounded by a lighter halo. Lewy bodies can also be found, although in lesser numbers, in some other CNS degenerative conditions and occasionally in the brains of nonparkinsonian elderly persons. In Parkinson's disease, Lewy bodies are most prominent in the substantia nigra, but they are also found in the locus caeruleus, nucleus basalis of Meynert, raphe nuclei, thalamus, and cerebral cortex. To the extent the cerebral cortex and the nucleus basalis of Meynert contain Lewy bodies or other forms of pathology, dementia may appear in some Parkinson's patients.

ETIOLOGY AND TREATMENT

A Toxin Can Cause a Syndrome Similar to Parkinson's Disease

Parkinsonism developed in a group of narcotic addicts who mistakenly injected themselves with the compound MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). MPTP is metabolized by brain MAO to MPP⁺. MPP⁺ is conveyed into dopamine nerve terminals by the dopamine transporter, which

normally acts to terminate dopaminergic neurotransmission by reaccumulating dopamine into presynaptic nerve terminals. The transporter also accumulates neurotoxins such as MPP⁺ that share structural features with dopamine.

Once inside dopaminergic cells, MPP⁺ accumulates within mitochondria, where it inhibits complex I of the mitochondrial respiratory chain, resulting in cell death. Decreased complex I activity has also been reported in naturally occurring Parkinson's disease, suggesting that the underlying mechanism of cell death may be similar to that documented in MPTP parkinsonism. Based on these processes, it can be appreciated why this toxin preferentially affects dopaminergic cells and why it causes a syndrome almost identical to that of ordinary Parkinson's disease.

In the laboratory, injection of MPTP into primates reliably produces parkinsonism. The remarkable resemblance of MPTP parkinsonism to naturally occurring Parkinson's disease raises the possibility that Parkinson's disease itself may be caused by a similar environmental toxin. Although MPTP itself has not been found in the environment, other potential toxins have been implicated. Epidemiologic studies have suggested that Parkinson's disease may be related to rural living, raising the possibility that agrichemicals may play a role in its causation. Some have proposed that a complex interaction of environmental and genetic factors may combine, resulting in degeneration of nigral neurons, subsequently leading to increasing mitochondrial respiratory failure and oxidative stress, which in turn may induce apoptotic cell death.

Monoamine Oxidase Inhibition May Benefit Patients with Parkinson's Disease

Drugs that inhibit the enzyme MAO block MPTP from inducing experimental parkinsonism by preventing the conversion of this protoxin to its active toxic form, MPP⁺. The fact that MAO inhibitors prevent the development of MPTP-induced parkinsonism is one factor that has suggested their use to prevent or retard the development of naturally occurring Parkinson's disease, especially if that condition is caused by a neurotoxin biochemically related to MPTP.

There is another mechanism through which MAO inhibitors may benefit the Parkinson patient. MAO is normally involved in the catabolism of dopamine. MAO-inhibiting drugs slow this process, reducing the associated generation of potentially toxic free

radicals, which may produce further nigral damage. Whether this effect of MAO-inhibiting drugs actually retards dopaminergic cell death and slows the progression of Parkinson's disease is not firmly established.

Dopaminergic Preparations Are the Most Effective Therapy for Parkinson's Disease

In the 1950s, it was discovered that dopamine is profoundly depleted in the striatum of patients with Parkinson's disease. This led to initial attempts to treat parkinsonian patients with small, orally administered dosages of levodopa, a dopamine precursor that, unlike dopamine itself, can cross the blood-brain barrier. Once inside the brain, it was reasoned, levodopa could be transformed to dopamine by the enzyme dopa-decarboxylase. These initial attempts were unsuccessful because the small dosages of levodopa employed were almost entirely metabolized to dopamine peripherally by dopa-decarboxylase in the liver and gut and therefore lost to the brain. Subsequent attempts in the late 1960s using much larger dosages were successful in allowing some levodopa to escape peripheral decarboxylation and enter the brain. It was soon apparent that this strategy was remarkably effective in reversing the symptoms of Parkinson's disease. Levodopa remains the mainstay of therapy for Parkinson's disease. It is now administered with a peripheral dopa-decarboxylase inhibitor, and almost no conversion to dopamine occurs outside the brain, allowing smaller amounts of levodopa to be administered.

More recently, a new class of medications termed catechol-*O*-methyl-transferase (COMT) inhibitors have been developed. These medications inhibit the conversion of levodopa to 3-*O*-methyl-dopa by COMT, thereby promoting its conversion to dopamine by aromatic amino acid decarboxylase within the CNS. These agents therefore increase or prolong the action of levodopa when coadministered.

Similar but less reliable improvement of parkinsonian symptoms can be achieved by the administration of dopamine agonists, such as pergolide or bromocriptine, and, more recently, pramipexole and ropinirole. These substances readily pass the blood-brain barrier and directly stimulate dopamine receptors, simulating the effect of dopamine. Because of concern regarding the potential complications associated with long-term levodopa use, dopamine agonists are being increasingly used in the early treatment of Parkinson's disease.

Severe Complications Can Occur After Years of Levodopa Therapy

After 5 years of levodopa therapy, approximately 50% of Parkinson's disease patients develop complications consisting of involuntary writhing, twisting movements of the extremities, trunk, and face, or episodes of sudden, transient, near-total loss of dopamine effect on their symptoms. The sudden loss of antiparkinsonian effect has been referred to as the *on-off effect*. Patients who experience this complication find that they may suddenly revert to total immobility as though someone had flipped a switch, followed by an equally sudden return to normal mobility. The unpredictability of these fluctuations in mobility can be extremely disabling. Both the on-off effect and the involuntary writhing movements, termed *dyskinesias*, that occur in patients with advanced Parkinson's disease are postulated to be related to the development of altered dopamine-receptor sensitivity. Whether these putative receptor changes are a function of the duration of the underlying disease or result from long-term nonphysiologic-receptor stimulation by dopaminergic drugs is unknown.

Tissue Transplantation May Improve the Symptoms of Parkinson's Disease

In the 1980s, there was considerable interest in transplantation of tissue into the CNS as a means of treating Parkinson's disease. Initial reports suggested improvement after transplantation of cells derived from the patients' own adrenal glands into the caudate nucleus. Cells from the adrenal medulla are metabolically capable of synthesizing dopamine, and it was reasoned that they could survive in the brain and elaborate this neurotransmitter. Although there were initial reports of improvement in patients who underwent this procedure, subsequent patients did not fare as well. The postmortem studies suggested that most transplanted cells did not survive.

A second wave of enthusiasm for cell grafting in treating Parkinson's disease has been generated by the early experience with the transplantation of tissue derived from human fetal mesencephalon and implanted into the caudate or putamen of patients. Careful study has shown that these cells do survive in the recipient's brain and effectively increase dopamine production, as demonstrated by positron emission tomography (PET). Unfortunately, consistent benefit has not yet been shown with fetal cell transplantation. However, it has been speculated that transplantation with higher numbers of fetal cells or in those with milder disease might still prove effective.

in the future. The use of cells other than fetal tissue that have been genetically modified to produce dopamine has also been proposed.

Surgical Therapies Can Improve Parkinson's Disease

Microelectrode recordings in Parkinson's disease patients have demonstrated abnormal activity in basal ganglia structures, most notably overactivity in the subthalamic nucleus (STN). Accordingly, various forms of ablative therapy of the STN or its efferent target, the internal segment of the globus pallidus, have been successfully employed to treat Parkinson's disease. Although thermocoagulation of

cells in these structures is possible, the preferred mode of cellular suppression is administration of an electrical stimulation to the targeted nucleus (deep brain stimulation).

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THE TYPICAL SYMPTOMS OF PERIPHERAL NEUROPATHY INCLUDE POSITIVE
 AND NEGATIVE PHENOMENA

NERVE-CONDUCTION TESTING IS USEFUL IN ANALYZING PERIPHERAL NEUROPATHIES

PERIPHERAL NEUROPATHY MAY DEVELOP ACUTELY OR CHRONICALLY

THERAPY FOR NEUROPATHIES CAN BE DIRECTED AT THE SYMPTOMS AND THE CAUSE
 SELECTED READING

1. PAIN IS A COMPLEX SENSORY EVENT

Pain can be a useful warning signal, an alarm that prevents us from damaging our bodies irreparably as we go about daily life. On the other hand, pain arising late in the course of disease or produced by

dysfunction of the nervous system has no survival value. Indeed, there is accumulating evidence that pain *per se* can have adverse health effects. For example, pain has been demonstrated in animal studies to inhibit immune function and to enhance tumor growth. In humans, appropriate pain management can be shown to enhance healing. Pain is thus an important clinical issue in its own right.

Although pain is part of everyday life, a clinically and scientifically useful definition of pain is not straightforward. This becomes particularly important when considering some of the types of pain with significant clinical impact. The International Association for the Study of Pain (IASP) therefore developed a consensus definition of pain that is now widely accepted. According to the IASP, *pain is an unpleasant bodily sensory experience commonly produced by processes that damage, or are capable of damaging, bodily tissue.* Thus, the stimulus is generally tissue damage produced by some sort of injury or disease. (Processes that are harmful or at least potentially harmful to the body are referred to as *noxious*.) This definition also emphasizes that pain is a sensory experience and leads to a distinction between pain and *nociception*, which refers to the neural mechanisms involved in detecting tissue damage. The primary reason for the distinction between pain and nociception is the two are often dissociated: a given noxious stimulus may or may not give rise to a pain sensation, and it is possible to have significant tissue damage with little pain. Conversely, there are some situations where pain, very intense and very real pain, occurs without any damage to tissue, or after healing is apparently complete. Finally, the definition recognizes that pain is multidimensional. Pain has an important emotional or *motivational* component, in addition to the discriminative component related to judgments about intensity, quality, and location. Even mild pain is unpleasant, and all organisms are highly motivated to escape from or avoid pain. Although the motivational aspect is the most troubling aspect of pain for most individuals, it is valuable in that it alerts the organism that something is wrong and provides an incentive to escape from the damaging event and to engage in needed recuperative behaviors. This aspect of pain also prompts us to avoid similar stimuli in the future.

2. SOMATOSENSORY PRIMARY AFFERENTS TRANSDUCE STIMULUS ENERGY AND TRANSMIT INFORMATION FROM THE PERIPHERAL TISSUES INTO THE CENTRAL NERVOUS SYSTEM

2.1. Somatosensory Receptors Are Specific in Their Response Properties

Somatosensory afferents are pseudo-unipolar neurons with cell bodies located in the dorsal root ganglion at each spinal segment. Each *primary afferent neuron* has two branches: one travels out to the peripheral tissues, and a second shorter central branch enters the spinal cord (Fig. 1). (Note that the cell bodies of afferents innervating the face reside in the trigeminal ganglion and project into the trigeminal system in the pons and medulla. Similar general principles apply to trigeminal as to spinal afferents, however, the subsequent discussion will focus on spinal systems.) The task of the primary afferents is to transduce stimulus energy into a form that can be used by the nervous system (i.e., action potentials) and to transmit that information into the central nervous system. The somatosensory system is unlike vision or audition in that the sensory afferent itself is the primary sensory transducer, without a separate specialized receptor cell. Different primary afferents are specialized to respond to different forms of stimulus energy. Thus, *low-threshold mechanoreceptors* are activated by mechanical stimuli and not at all by heating or cooling, whereas *thermoreceptors* respond to innocuous warming or to cooling but not to mechanical stimuli. *Nociceptors* are more diverse but are activated by various stimuli that have in common the ability to damage tissue. Adequate stimuli for a nociceptor may include intense mechanical,

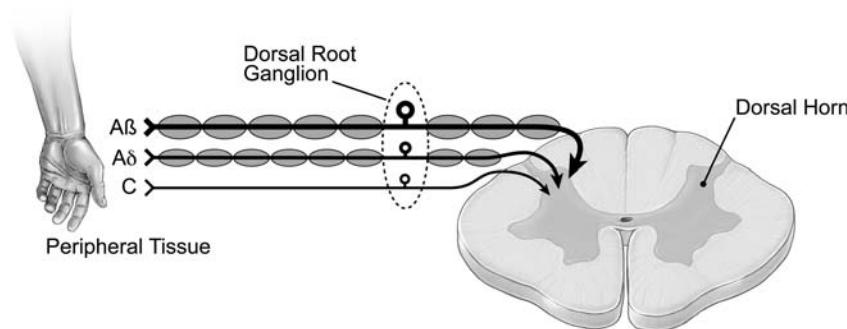


Fig. 1. Schematic view of somatic primary afferent axons in a cutaneous nerve. Pseudo-unipolar cells with cell bodies in the dorsal root ganglion send a peripheral branch to the skin and a central branch into the dorsal horn of the spinal cord via the dorsal root. Afferents can be divided into A β fibers, which have large myelinated axons, A δ fibers, which have smaller myelinated axons, and C fibers, which have small, unmyelinated axons.

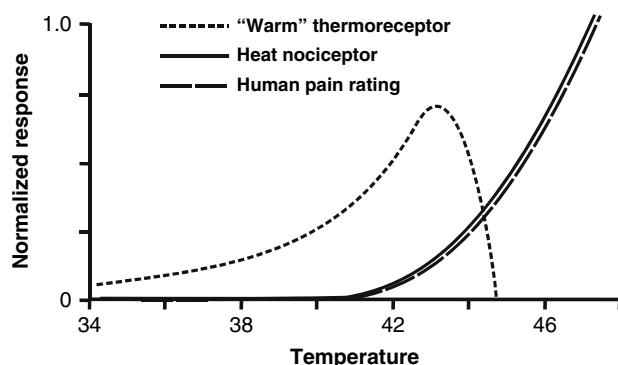


Fig. 2. Coding of innocuous and noxious heat by thermoreceptors and thermal nociceptors, respectively. Thermoreceptors exhibit a temperature-related activation as the skin is warmed above a holding temperature of about 30–34°C to about 42°C, the threshold for heat pain in humans. Activity then drops dramatically as temperature is increased into the noxious range. In contrast, heat nociceptors are not activated by innocuous warming but show a temperature-related increase in activity that parallels human pain rating when the temperature is above 42°C.

thermal, and/or chemical insults. The difference between a thermoreceptor responsive to innocuous warming and a nociceptor responsive to intense heat is illustrated in Fig. 2. The thermoreceptor shows a gradual increase in activity as skin temperature is increased from a normal skin temperature around 30°C to about 42°C, the point at which the stimulus would be perceived as painful by humans and at which a prolonged stimulus can produce damage. Firing of the thermoreceptor is then depressed. In contrast, the threshold for a thermal nociceptor is around 42°C, and the increased firing of the afferent as skin temperature increases parallels human ratings of the pain elicited by the thermal stimulus.

Primary afferents vary both in terms of axon diameter and whether or not they are myelinated (Table 1).

Axons going to skin are divided into A β , A δ , and C classes. The largest fall into the A β group, and because of their large size and myelination, they conduct action potentials rapidly. The smaller myelinated axons are termed A δ fibers, and these conduct more slowly than the A β fibers. Unmyelinated afferents are of extremely fine caliber, and accordingly conduct very slowly (<2 m/s). With electrical stimulation, the amount of current required to activate the different classes varies inversely with diameter. The largest fibers thus have a low electrical threshold and are activated relatively easily with electrical stimulation. A much higher current is required to activate C fibers. The different conduction velocities are reflected in the *compound action potential*, which is the summed activity that can be recorded from a peripheral nerve when it is stimulated electrically. The earliest peak recorded in the compound action potential represents discharges of the A β afferents, which travel rapidly down the axons to the recording site. The second peak is due to activity in the A δ fibers, and the final, much later and more dispersed peak represents discharge of the C fibers.

Table 1 also outlines a parallel classification scheme using roman numerals that is widely employed for afferents innervating joints and muscle. Muscle afferents include a class of very large diameter axons (Ia axons innervating muscle spindles and Ib axons innervating the Golgi tendon organs). Axons of this size are not found in cutaneous nerves. Visceral afferents are classified using the same system as cutaneous afferents.

At a gross level, the function of the different cutaneous afferents can be correlated with morphology and conduction velocity. The best studied low-threshold mechanoreceptors are large myelinated afferents conducting in the A β range. These afferents have encapsulated endings and are specialized for rapidly

Table 1
Classification of Somatic Primary Afferents by Size and Myelination

Cutaneous	Muscle	Viscera	Fiber diameter (μm)	Conduction velocity (m/s)	Functional correlates
Myelinated	Ia, Ib	A δ	12–20	80–120	Muscle spindle afferents, Golgi tendon organ
					Low-threshold mechanoreceptors
					Nociceptors, innocuous cooling, mechanoreceptors
Unmyelinated	C	IV	C	0.2–1.5	Nociceptors, innocuous warming, mechanoreceptors

or slowly adapting responses to innocuous mechanical stimuli. Additional low-threshold mechanoreceptors (e.g., hair afferents) are found within the A δ and C classes, as are thermoreceptors. Those thermoreceptors responding to innocuous cooling generally fall into the A δ group and those activated by warming into the C group. Like thermoreceptors, nociceptors are found primarily among the small-diameter primary afferents with slower conduction velocities, the A δ and C-fiber groups. Many nociceptors are *polymodal*, that is, they respond to both intense heat and intense mechanical stimuli, and in some cases to chemical irritants. Others are more selective, responding only to mechanical or chemical stimuli. There are in addition large numbers of nociceptors that have extremely high thresholds for activation in healthy tissues. However, these unresponsive axons become highly sensitized after inflammation and are thus sometimes referred to as *silent* or *sleeping* nociceptors, because they “awaken” after injury or inflammation.

2.2. Specific Response Properties of Somatosensory Primary Afferents Lead to Specificity of Function

The importance of small-diameter fibers for pain sensation was originally inferred from experiments using electrical stimulation of whole nerves. These experiments demonstrated that high stimulus currents, sufficient to activate the smaller-diameter fibers, were required to produce painful sensations. Conversely, selective ischemic block of the activity in the largest afferents interfered with tactile sensibility, but not pain or thermal sensation. The functional specificity of individual primary afferents has since been confirmed in elegant experiments using microneurography and intraneuronal microstimulation in conscious human subjects. In these experiments, the discharges of an individual sensory afferent are identified by recording with a microelectrode (*microneurography*). The response properties of the axon are then defined using natural stimulation of its receptive field on the skin (e.g., brushing, pinching, cooling or warming, or intense heating). The same electrode is then used to stimulate that axon (*intraneuronal microstimulation*). Investigators taking this approach have shown that electrical stimulation of a *single* A β low-threshold mechanoreceptor causes the subject to report a sensation of tapping, vibration, or sustained pressure (depending on the type of mechanoreceptor stimulated and the stimulus frequency). These sensations are perceived as arising from the afferent’s *receptive field*, the area of skin from which tactile stimulation activates the fiber.

Stimulation of a large A β afferent does not evoke pain sensations under normal conditions, even when stimulus frequency is increased to drive the fiber at a high rate. In contrast, direct electrical stimulation of a single A δ nociceptor produces a sensation of pain, usually sharp or pricking in character. Although stimulation of individual C-fiber nociceptors has not been possible because of their small size, stimulation of a small bundle of these fibers produces a dull burning pain or itch. These findings demonstrate that activity in the low-threshold mechanoreceptors does not contribute to pain, whereas activity in a single A δ nociceptor is sufficient to produce pain. (Note that while this is invariably true under normal conditions, we shall see later that there are persistent pain states in which activating the non-nociceptive tactile afferents evokes significant pain.) Stimulation of C fibers produces pain or itch, although we cannot say whether activity in one or many C fibers is required to produce a sensation.

2.3. Chemical Mediators of Nociception

Our understanding of how damage to tissue results in activation of the primary afferent nociceptors has been the focus of a great deal of research but is still incomplete. However, there are a number of chemicals that are released or synthesized when tissue is damaged. Some of the constituents of this “chemical soup” are listed in Table 2. Receptors and channels targeted by these compounds are now being defined. Many of these substances are known to activate nociceptors and induce pain when applied to human volunteers. Others do not by themselves activate the nociceptors, but *sensitize* them, causing them to be more responsive to other inputs. As will be discussed in more detail below, the resulting sensitization of the primary afferent nociceptors is a major factor in enhanced pain after injury or inflammation.

2.4. Small-Diameter Afferents, Including Nociceptors, Are Rich in Neuropeptides

With the physiologic identification of nociceptive primary afferents came the hope that they would release a unique neurotransmitter. Interest in this idea was high, because a transmitter specific to nociceptors would provide an accessible and selective target for potential pain treatments. Unfortunately, this turned out not to be the case.

The major fast excitatory transmitter in the small afferents is glutamate, an excitatory amino acid neurotransmitter that is also expressed by large

Table 2
Chemical Mediators of Nociception and Inflammation After Damage to Tissues

Leak from damaged cells or stimulated release:

- *Ach*: released from damaged cells, causes pain.
- *Protons*: in-migrating leukocytes secrete lactic acid; key aspect of inflammatory “soup” is low pH.
- *5HT*: released from platelets, intradermal application produces pain, activates nociceptors.
- *ATP*: application produces pain, activates nociceptors.
- *Histamine*: released by mast cells, produces itch not pain, but along with 5HT, BK (Bradykinin), stimulates synthesis of prostaglandins.

Synthesized locally by enzymes from substrates released by damage or that enter area as part of inflammatory process (e.g., leukocyte migration):

- *Bradykinin*: synthesized from protein precursor in plasma (kininogen), activates nociceptors to produce pain, sensitizes nociceptors to other inputs (including heat), triggers other elements of inflammatory process (including synthesis of prostaglandins and release of cytokines).
- *Prostaglandins*: synthesized from arachidonic acid after injury. Contributes to inflammation by inducing vasodilation and plasma extravasation, attracts immune cells. Sensitizes nociceptors.

Released by activated nociceptors themselves:

- *Substance P*: acts on other afferent fiber terminals to activate and/or sensitize, contributes to inflammation by causing vasodilation, plasma extravasation. Stimulates mast cells to release histamine.
- *CGRP (calcitonin gene-related peptide)*: contributes to inflammation, dilates arterioles, synergizes with substance P in producing plasma extravasation.

mechanoreceptor afferents. However, the small-diameter afferents (both nociceptors and thermoreceptors) are remarkable in also expressing an array of neuropeptides, among them substance P, calcitonin gene-related peptide, vasoactive intestinal peptide, and somatostatin. Indeed, many of these afferents colocalize several peptides as well as glutamate. These peptides are released along with glutamate.

The best studied of the many neuropeptides localized in small-diameter afferents is substance P, an 11-amino-acid neuropeptide that originally received considerable attention from investigators attempting to identify the “pain transmitter” in the spinal cord. However, blocking the action of substance P released from primary afferent nociceptors does not produce a potent analgesia. This indicates that although substance P may play a role in nociception, it is not the sole contributor. It is now recognized that the corelease of glutamate and various peptides is an important part in the signaling of nociceptive information.

2.5. Primary Afferents Play an Active Role in Promoting Inflammation at a Site of Injury

It is also worth noting that *most* of the peptide manufactured by the primary afferent neurons is actually transported out to the periphery, rather than centrally into the dorsal horn. For example, about 80% of the substance P produced by cells in the dorsal root ganglion is transported out to the tissues. These peripherally released peptides have

been shown to promote inflammation and contribute to sensitization and activation of the primary afferent terminals (Table 2). Thus, the primary afferent neurons are not mere passive sensors for damage. Rather, they participate actively in dynamic alterations of peripheral sensory sensitivity and contribute to inflammation as part of the repair process.

3. NOCICEPTIVE SENSORY NEURONS PROJECT TO THE DORSAL HORN

3.1. The Pattern of Projection and Termination of Somatosensory Afferents Within the Spinal Cord Is Highly Ordered

Somatosensory primary afferents travel through the dorsal root to enter the spinal cord (or in the case of the head and neck, the trigeminal nucleus in the medulla, which can be considered the medullary homologue of the spinal somatosensory processing circuitry). The pattern of branching and termination within the dorsal horn shows a high level of anatomic ordering that can be related to function (Fig. 3). Upon entering the cord, the small fibers may travel within the white matter adjacent to the entry zone for several segments, but then terminate within the dorsal horn in two regions: the superficial layers (I and II) and lamina V. In contrast, the low-threshold mechanoreceptors course around the medial edge of the dorsal horn and send branches rostrally toward the

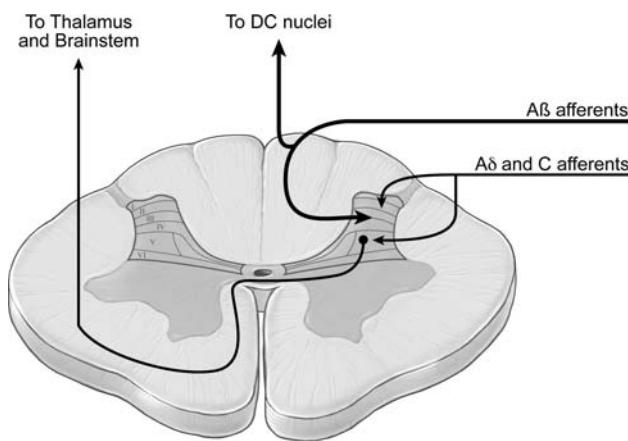


Fig. 3. Organization of somatosensory afferent terminations in the dorsal horn. As axons approach the spinal cord in the dorsal root, axons segregate by size, so that the A β afferents move dorsally and medially, whereas the A δ and C afferents shift laterally. Large mechanoreceptive afferents then project through the dorsal columns to the medulla and may send a branch into the middle layers of the dorsal horn (laminae III and IV). The smaller afferents terminate superficially (laminae I and II) and in the neck of the dorsal horn (lamina V).

medulla in the dorsal columns and into the middle layers of the dorsal horn (laminae III and IV).

3.2. Nociceptive Dorsal Horn Neurons Include Some That Are Nociceptive Specific and Others That Respond to Both Innocuous and Noxious Stimulation

Given the ordered distribution of primary afferents, it should not be surprising that dorsal horn neurons activated by tissue damage are found in the superficial layers and more deeply in lamina V. Dorsal horn nociceptive neurons can be divided into two broad classes: nociceptive specific (NS) and wide-dynamic range (WDR; Fig. 4). NS neurons are excited only by nociceptive primary afferents. They do not respond to brush or even moderate pressure. They generally have small receptive fields (e.g., only a toe and part of foot), which suggests that they could be very important in coding the location of a noxious stimulus. WDR neurons receive input from low-threshold as well as nociceptive primary afferents and show a graded increase in firing rate related to stimulus intensity. Thus, unlike primary afferents, they respond to stimuli over a wide range of intensities, both innocuous and noxious. The receptive fields of WDR neurons tend to be larger than those of NS neurons, suggesting that WDR neurons do not contribute substantially to location coding. However, the firing rate of WDR cells is generally

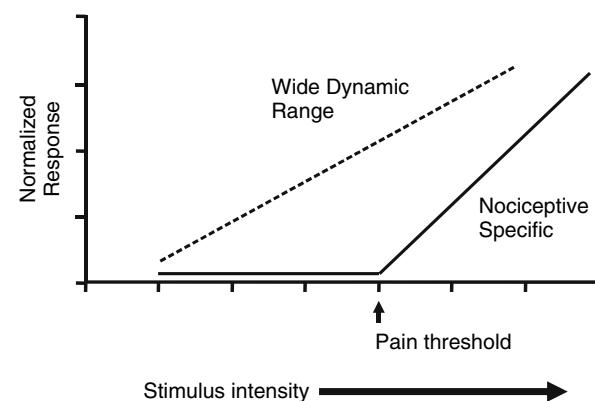


Fig. 4. Dorsal horn nociceptive neurons include nociceptive specific (NS) cells, which respond only to stimuli with intensity in the noxious range, and wide dynamic range (WDR) cells, which are activated by both innocuous and noxious stimuli, with a graded increase in activity.

well correlated with both stimulus intensity and subjective pain intensity in humans, so they are thought to code stimulus intensity.

4. PARALLEL ASCENDING PATHWAYS UNDERLIE DISTRIBUTED PROCESSING OF NOCICEPTIVE INFORMATION

4.1. Nociceptive Dorsal Horn Neurons Contribute to the Anterolateral System

Some of these nociceptive dorsal horn neurons (NS and WDR) are interneurons (i.e., involved in processing either within the same segment or at other levels within the spinal cord) but many are projection neurons, sending their axons up to the brain. The axons cross to the contralateral side and ascend along with axons from thermoreceptive dorsal horn neurons in the white matter of the anterolateral quadrant as part of the *anterolateral system* (Fig. 5). This is in contrast with projections of large-diameter afferents, which are via the dorsal column ipsilateral to the site of entry (Fig. 3). Hemisections of the spinal cord thus result, at least in the short-term, in a loss of fine touch *ipsilaterally* and pain and temperature *contralaterally*. It should be noted that there is a small contingent of anterolateral system fibers that ascend ipsilaterally. This ipsilateral projection seems to make only a small contribution to sensation under normal conditions but may contribute to intractable pain that sometimes develops after section of the contralateral anterolateral quadrant.

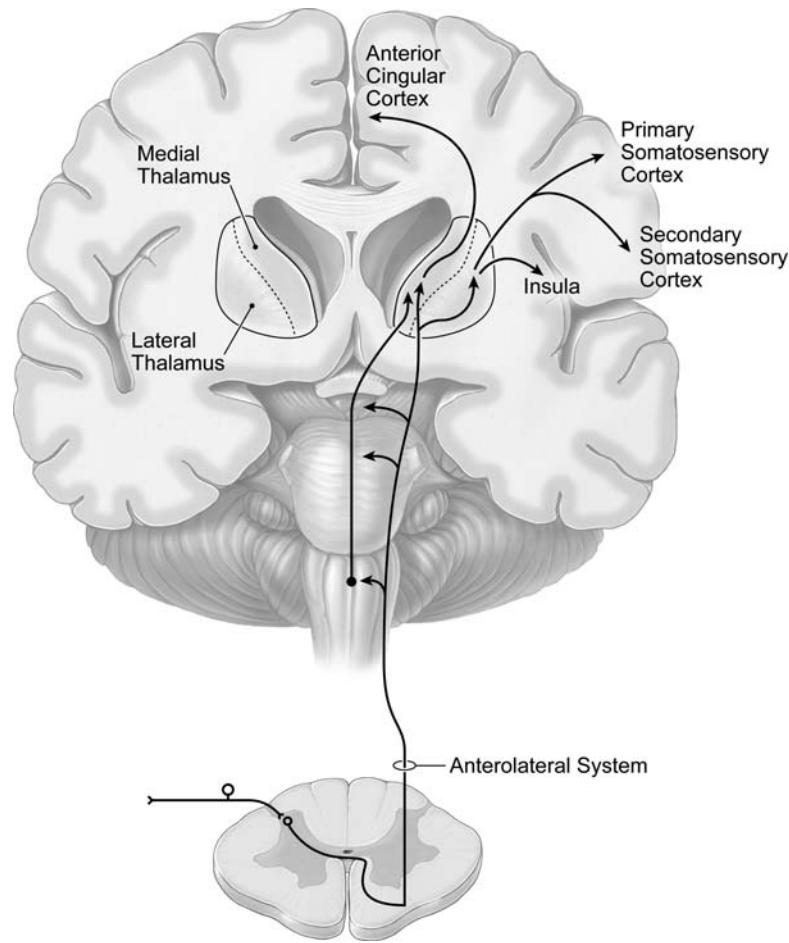


Fig. 5. Organization of the anterolateral system. Spinothalamic projections to lateral and medial thalamus as well as spinoreticular projections to brain-stem reticular formation are shown.

4.2. Parallel Pathways Within the Anterolateral System Form the Basis for Nociception

The anterolateral system has a number of targets in the brain. Among the most important is a direct spinothalamic projection to the lateral and medial thalamus (Fig. 5). Lateral spinothalamic targets (ventral posterolateral nucleus as well as more posterior and inferior regions that have not been as well studied) in turn project to primary and secondary somatosensory cortex and to the insula. These projections are believed to convey information needed for discriminative analysis of painful stimuli and play an important role in monitoring of the “state of the body.” At least as a first approximation, information related to the motivational component of pain is thought to involve projections to the medial thalamus, both direct and relayed through the brain-stem reticular formation. Some medial thalamic nuclei project to anterior cingulate cortex, a region considered to be closely linked to

limbic processing of sensory information and decision-making. Recent functional imaging studies in humans confirm activation of the insula, anterior cingulate, and somatosensory cortex during painful stimulation. Spinoreticular projections that terminate in brain-stem reticular formation are thought to contribute to arousal and can also access medial thalamic structures indirectly (Fig. 5). Finally, the anterolateral system also includes spinal projections directly to limbic and striatal forebrain structures, including amygdala and hypothalamus. These are presumed to play some role in sensory processing and/or neuroendocrine and autonomic adjustments needed to deal effectively with damage to the body.

Our understanding of the central neural mechanisms of pain sensation has increased substantially in recent years. A broad framework in which a crossed anterolateral system served as the pathway for sensations of pain can be traced back to clinical and

experimental observations made more than a century ago. Indeed, as early as 1911, Head and Holmes suggested that discriminative and affective components of pain sensation were mediated by the lateral and medial thalamus, respectively. A role for cortical structures in pain sensation was generally discounted, as cortical lesions rarely altered pain sensation in patients. The recognition that the spinoreticular and spinothalamic projections can access and activate multiple cortical structures, as well as recent evidence that the anterolateral system has direct projections to forebrain structures, demand a more complex view. Parallel ascending pathways target different areas in the brain stem, thalamus, and forebrain. These projections likely process different aspects of the noxious stimulus and interact in a dynamic fashion to give rise to sensation. This fact, more than any other, probably accounts for our inability to produce durable analgesia by ablative central lesions. Whatever tract is cut or brain region destroyed, there are always other circuits, redundant and plastic, that can reconstitute the nociceptive process.

5. VISCERAL NOCICEPTION

5.1. *Stimuli That Give Rise to Pain Sensation Depend on the Tissue Stimulated*

Although most clinically significant pains arise from deep somatic structures or viscera, the discussion up to now has been focused upon neural mechanisms of pain arising from the skin. That is at least in part because the skin is relatively easy to study, and we consequently know much more about it. However, the skin is a specialized tissue, important both as a sensory organ and as part of our defense against the outside world. Pain from deep structures, particularly the viscera, thus differs in a number of ways from that arising from skin.

For one thing, the effective stimuli are different. Extreme damage to a visceral organ does not necessarily give rise to pain. Indeed, surgeons have long known that it was possible to cut or cauterize visceral tissue without producing pain. This astonishing observation led to the idea that there was no such thing as pain from visceral structures, and pain that seemed to the patient to be derived from viscera actually arose because somatic structures were somehow involved. However, it later became clear that pain does arise from most visceral structures and that appropriate stimuli, which include distention of hollow organs, ischemia (e.g., the heart), and inflammation, are important sources of visceral pain.

Another difference is that spatial summation seems to be important in visceral pain, although this is not the case with pain from skin. We all recognize that pinprick is painful, although the area stimulated is quite small. With visceral structures, it is necessary to stimulate over a larger area, which may explain why visceral inflammation is painful, whereas more localized cutting or pinching is not.

5.2. *Visceral Pain Is Poorly Localized*

One of the real peculiarities of pain from visceral organs is that it is poorly localized and often perceived as arising in a body region remote from the actual pathology. The most commonly cited example is angina pectoris, in which a significant number of patients report pain in the left chest and shoulder or upper arm. This remote pain is called *referred pain*, and it can be felt either in other deep structures or in skin. The pattern of referral is sometimes characteristic for a particular structure and can be a helpful diagnostic aid.

5.3. *Convergence of Somatic and Visceral Information in the Dorsal Horn Explains Referred Pain from Visceral Structures*

Mechanisms of referred pain have been intensely debated. It should be recognized that the primary afferents innervating viscera are small diameter ($A\delta$ and C fibers) and project primarily to superficial dorsal horn and deep dorsal horn, skipping laminae III and IV. They thus resemble cutaneous nociceptors in axon diameter and central projection. However, the innervation of the viscera is generally much less dense than that of the skin and the projections within the dorsal horn more diffuse. Visceral afferents show extensive spread along the rostro-caudal axis and may even project to the contralateral dorsal horn. In addition, visceral afferents target dorsal horn neurons that also receive input from the skin or from musculoskeletal tissues. Thus, there are no specific “visceral” neurons in the dorsal horn. This suggests that referred pain is due to convergence of inputs in the dorsal horn (*convergence-projection theory*). When the brain receives a signal from a dorsal horn neuron, it has no way of knowing the true source, and mistakenly “projects” the sensation to the skin or another somatic structure (Fig. 6). The projection of visceral input to skin rather than vice versa is likely due to the fact that there are many neurons with strictly cutaneous input that bias supraspinal processing when active.

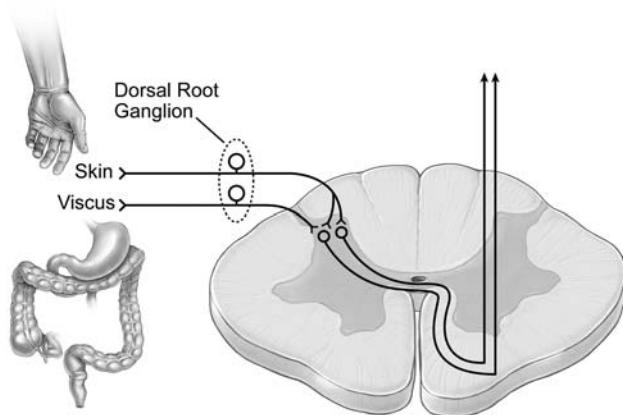


Fig. 6. Convergence-projection theory explains referral of visceral pain to skin. Dorsal horn neurons with input from visceral structures also receive input from cutaneous afferents.

6. PAIN PROCESSES IN INJURED OR INFLAMED TISSUE

Up to this point, we have been talking for the most part about brief stimuli delivered to normal tissue that do not produce significant damage or inflammation. The pain that results under these circumstances is sometimes referred to as “normal” pain, and mildly painful stimuli are certainly encountered frequently as part of everyday life. In contrast, pain experiences that induce significant distress usually involve more serious destructive processes. In these situations, there is often a lasting tenderness and hypersensitivity. This increase in pain sensation is loosely referred to as *hyperalgesia* and can involve a decrease in threshold (so that normally innocuous stimuli such as a light touch or gentle warmth are now perceived as

painful, a state referred to as *allodynia*) and increased sensation evoked by stimuli that would normally cause at least some pain.

Hyperalgesia, the increase in pain *sensation* after injury, is caused by *sensitization* of nociceptive processing circuits, peripherally and centrally. However, when considering the mechanisms underlying hyperalgesia after injury, it is important to distinguish between *primary hyperalgesia*, which is exaggerated sensitivity in the injured tissue, and *secondary hyperalgesia*, which refers to the increased sensitivity in the surrounding area (Fig. 7A). This distinction between primary and secondary hyperalgesia is significant because afferents innervating the injured region, but not the nearby areas, are sensitized.

6.1. Primary Hyperalgesia Involves Increased Sensitivity and Responsiveness in the Primary Afferent Nociceptors

Primary hyperalgesia is explained by increases in the sensitivity of the primary afferent nociceptors (Fig. 7B). Both A δ and C-fiber nociceptors are sensitized after injury and inflammation. Thresholds for activation are lowered, and the afferents display an increased response to suprathreshold stimuli. These afferents can also develop spontaneous activity and show prolonged afterdischarges so that the neuronal response outlasts the stimulus. In addition, as already noted, many nociceptors are silent or “sleeping” under normal conditions but become quite responsive to mechanical and thermal stimuli when the tissue is inflamed.

Mechanisms for peripheral sensitization involve the chemical “soup” that develops in injured tissues

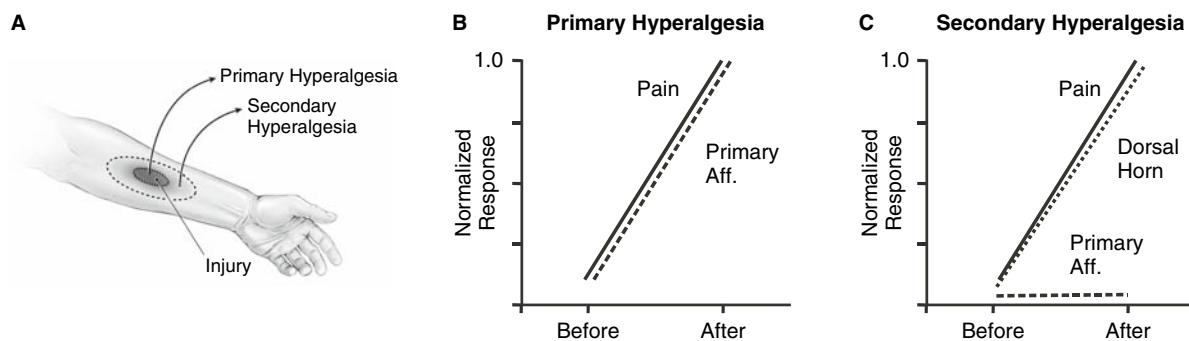


Fig. 7. Mechanisms of primary and secondary hyperalgesia. **(A)** Hyperalgesia within an area of injured tissue is referred to as primary hyperalgesia, and that in surrounding tissue is called secondary hyperalgesia. **(B)** Pain rating and activity in primary afferents before and after injury to the tissue. Sensitized primary afferents explain increased pain in the injured region. **(C)** Pain rating and activity in primary afferents and dorsal horn neurons with receptive fields in the area of secondary hyperalgesia. Primary afferents innervating the area of secondary hyperalgesia are not sensitized, whereas dorsal horn neurons responding to stimulation of that area show increased responsiveness (*central sensitization*).

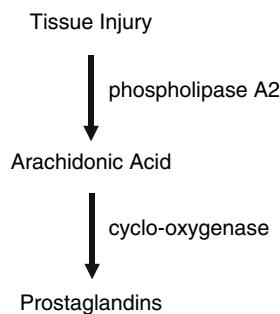


Fig. 8. Prostaglandins produced from membrane lipids after tissue injury. Aspirin and related analgesic drugs produce their effect by inhibition of cyclooxygenase.

(Table 2). Bradykinin, formed from a plasma precursor, directly activates the afferent terminal and also sensitizes it to other inputs. A number of these compounds do not excite the nociceptor directly, but will cause it to become sensitized. One effect of substance P released from the peripheral nerve terminals is to sensitize the terminals, making them more responsive to other components of the inflammatory soup. Prostaglandins, formed from membrane lipids by the actions of phospholipase A2 and cyclooxygenase (Fig. 8), also sensitize primary afferent terminals. Aspirin and related compounds reduce pain in part by blocking cyclooxygenase, thus preventing the formation of prostaglandins (that is, these drugs are cyclooxygenase [COX] inhibitors). Indeed, thermal sensitivity in inflamed tissue may be increased to the point that normal body temperature becomes sufficient to activate nociceptive afferents and produce pain!

Increased input to dorsal horn nociceptive neurons from sensitized primary afferents causes the dorsal horn neurons themselves to be sensitized. Thus, the hyperexcitability of the primary afferents propagates through the CNS nociceptive pathways, resulting in primary hyperalgesia.

6.2. Secondary Hyperalgesia Is Accounted for by Central Sensitization

Secondary hyperalgesia does not seem to be explained by primary afferent sensitization, because the properties of afferents with receptive fields in the area of secondary hyperalgesia are essentially unchanged. The significant alteration underlying hyperalgesia in tissue surrounding the injury instead seems to be in the dorsal horn (Fig. 7C). Thus, secondary hyperalgesia is explained not by sensitization of the primary afferents but by *central sensitization*. Studies of dorsal horn neurons with receptive fields in

an area of secondary hyperalgesia show lowered thresholds, with increased responses to frankly noxious stimuli. Many of these neurons also develop spontaneous activity. Changes are most pronounced in the wide dynamic range neurons, and the circuitry in the spinal cord is sensitized to the extent that inputs conveyed by large-diameter low-threshold mechano-receptor afferents become sufficient to elicit pain (although you will recall that this is not the case under normal conditions). Sensitization of dorsal horn neurons that do not themselves receive direct input from the injured tissue presumably reflects connections within the dorsal horn and a “spread” of sensitizing influences (e.g., neuropeptides) from areas that do receive a direct afferent connection from the injured region.

7. REGULATORY MECHANISMS: GATE CONTROL AND DESCENDING MODULATION

Nociceptive information is not imposed on a passive nervous system. It is subject to a number of control mechanisms. These can be divided into segmental mechanisms (i.e., within the spinal cord) and descending controls, which are exerted on the dorsal horn from the brain.

7.1. Segmental Control Mechanisms Include That Described by Gate Control Theory

The best-known example of segmental control mechanisms is the *gate control* proposed by Melzack and Wall in 1965. Their theory was based on the observation that when conduction in large-diameter fibers is blocked (e.g., by pressure that interferes with conduction in myelinated fibers without affecting unmyelinated axons), just about any stimulus, from light touch to pinch, gives rise to a very unpleasant tingling, burning pain. One way to explain this finding would be if activation of large tactile afferents activated an inhibitory interneuron, or “pain gate,” that blocked transmission of an ascending message giving rise to pain. Although the specific details of the gate control theory have not stood the test of time, the general idea that input conveyed over large-diameter, low-threshold afferents can interact with processing of nociceptive information does have some validity. A circuit something like that proposed by Melzack and Wall is probably the basis for some treatments used clinically, such as TENS (transcutaneous electrical stimulation). TENS involves electrical stimulation of the skin at an intensity that activates

large-diameter fibers (which have a lower threshold for electrical activation than do smaller fibers). Use of TENS is particularly widespread in treatment of musculoskeletal pain states.

7.2. Brain-Stem Control of Spinal Nociceptive Processing

The magnitude of the pain produced by any given noxious stimulus also depends upon the individual and an array of situational and behavioral factors. The important influence of cognitive and emotional factors on pain has been recognized for centuries, but the earliest systematic analysis of this variability was probably that provided by Sir Henry Beecher. He described soldiers wounded in World War II who experienced much less pain than would have been expected from their injuries. Subsequent psychophysical studies have clearly demonstrated that pain experience in humans and animals can be influenced systematically by arousal, attention, learning, fear, and stress.

Although the influence of psychological factors in modulating pain can impede both scientific study and treatment of pain, we now know that these variations in pain sensation have an understandable neural basis. The brain possesses central modulatory circuits that are specific for pain. This idea of central modulation is relatively recent and usually traced back to the observation that electrical stimulation in the midbrain periaqueductal gray of rats inhibited responses to stimuli that would have been expected to produce pain behaviors in normal animals. The significance of this observation, which appeared in *Science* in 1969, was in pointing to pain modulation as a specific function of the central nervous system. Although a number of brain systems are now known to regulate spinal nociceptive processing, the best studied and probably functionally most significant pain modulating network has links in the midbrain periaqueductal gray and rostral ventromedial medulla (Fig. 9). Activation of the periaqueductal gray in turn activates neurons in the rostral medulla that project to the dorsal horn at all levels of the cord and influence nociceptive processing at that level. The periaqueductal gray has dense reciprocal connections with limbic forebrain structures such as the amygdala and hypothalamus. These connections connect higher neural processes with pain regulation.

This descending modulatory system is also an important substrate for opioid analgesia. Local administration of opioids in either the periaqueductal gray or the rostral ventromedial medulla produces

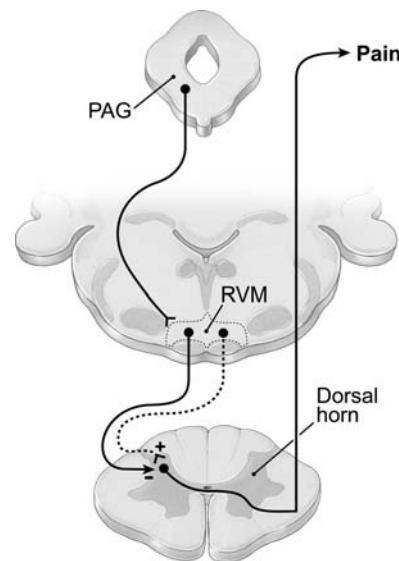


Fig. 9. Brain-stem pain-modulating network has links in the midbrain periaqueductal gray (PAG) and rostral ventromedial medulla (RVM). The RVM, which includes the nucleus raphe magnus and adjacent reticular formation, receives a large input from the PAG. The RVM in turns projects to the dorsal horn, primarily to the superficial layers and lamina I, where it can influence processing of nociceptive information. The RVM includes two populations of cells, *off-cells*, which inhibit nociception, and *on-cells*, which facilitate nociception at the level of the dorsal horn.

analgesia, and studies at the single cell level demonstrate that this is due to the ability of opioid analgesics to activate pain-inhibiting neurons within the rostral medulla. This system is also responsible for the inhibition of pain by various stressors. Interestingly, this descending control system has now been shown to contribute to *enhanced* nociceptive processing under some conditions, for example during illness or inflammation. The neural basis for bidirectional control of nociception is two classes of neurons in the rostral medulla: *off-cells*, which are activated by opioids and *inhibit* nociceptive processing, and *on-cells*, which are inhibited by opioids and *promote* nociceptive processing.

8. PAIN DUE TO INJURY TO PERIPHERAL OR CENTRAL NERVOUS SYSTEM

We have so far been considering *nociceptive pain*, that is, pain produced, or at least triggered by, tissue injury. There are, however, situations in which the source of pain is not in the tissue (where it is “perceived” to be) but due to damage to the nervous system itself, either the peripheral nerve or nociceptive

processing pathways in the spinal cord and brain. Damage can be due to trauma, (e.g., peripheral nerve or spinal cord injury, brachial plexus avulsion, or amputation) metabolic disorders (e.g., diabetes), infection (e.g., herpes zoster and shingles), or stroke. Various terms have been used to refer to pain after damage to the nervous system rather than the tissue. The most general is *neuropathic*, as it simply denotes pain arising from injury to neural structures.

8.1. Features of Neuropathic Pain Distinguish It from Pain Produced by Tissue Injury

There are many neuropathic pain syndromes (examples include postherpetic neuralgia, diabetic neuropathy, complex regional pain syndromes, spinal cord injury pain, phantom limb pain, and central pain), and the character of pain in these syndromes is quite distinct from pain produced by injury to non-neuronal tissues. Neuropathic pain is persistent, generally burning or with a “shooting” or “electric” quality. There is often extreme sensitivity to mechanical or thermal stimuli, so that everyday events such as the brush of clothing on the skin can be painful. Paradoxically, ongoing pain and heightened sensitivity can be perceived as arising from a region of sensory deficit.

Mechanisms underlying these painful dysfunctions of the nervous system remain poorly understood, and in most neuropathic syndromes there are probably multiple pathologic processes at work. Nevertheless, we can consider mechanisms involving abnormal activity in primary afferents, those due to sensitization in central pathways, and efferent mechanisms involving the sympathetic nervous system.

8.2. Damaged Primary Afferent Display Abnormal Activity

Normally, action potentials arise only at the peripheral terminals of primary afferents. However, when an axon is cut, new processes grow out toward the original target tissue. These sprouts often end up forming a tangle called a *neuroma*. The axon sprouts within the neuroma are abnormally thin, and without the usual Schwann cell sheath. These morphologic abnormalities are associated with altered physiology, so that there is spontaneous activity and sensitivity to mechanical stimulation. Tapping the neuroma thus produces a sensation. Abnormal activity in these damaged afferents is called *ectopic*, because it originates at an abnormal site, not at the terminal region, as is usually the case. Ectopic activity after injury may

also arise from the region around the cell body in the dorsal root ganglion. Wherever it arises, ectopic activity will be interpreted by central circuits as reflecting peripheral events, and it may contribute to a form of central sensitization, analogous to that underlying secondary hyperalgesia.

8.3. Central Factors in Neuropathic Pain Include Central Sensitization

We discussed above how injury and inflammation of non-neuronal tissue can drive changes in dorsal horn and supraspinal pathways referred to as *central sensitization*. Similar processes contribute to neuropathic pain when aberrant activity in primary afferents triggers and maintains heightened excitability in the CNS. This has been best documented in the dorsal horn, where nociceptive neurons, particularly wide dynamic range neurons, show increased spontaneous firing, enlarged receptive fields, and abnormal afterdischarges after injury to the peripheral nerve. Changes in the dorsal horn also carry through to higher brain regions. For example, electrical stimulation in thalamic regions that receive input from the spinothalamic tract is likely to evoke reports of pain in patients with chronic pain, but only cooling or other thermal sensation in patients without chronic pain. The descending modulatory system is also altered, so that the pronociceptive modulatory output from the brain stem overwhelms the antinociceptive inhibitory output.

It is relatively easy to understand how aberrant activity in injured afferents might give rise to an expanding cascade of altered central circuits that ultimately leads to neuropathic pain. The paradoxical pain that arises after *loss* of input is more difficult to explain. Pain in a phantom limb after amputation is a clear-cut example, but loss of input contributes to neuropathic pain in other situations that might be less obvious, for example, postherpetic neuralgia (where at least some dorsal root ganglion neurons die), after spinal cord injury (where dorsal horn neurons are lost), or after a stroke involving the spinothalamic tract or its targets in the thalamus (*thalamic pain*). Notably, the onset of pain in these situations may be very soon after the lesion but can be delayed for up to several months.

It is assumed that pain in these cases represents an attempt by the denervated neurons to compensate for lost inputs, resulting in a state analogous to *denervation hypersensitivity*. This is a state of hyperactivity that many central neurons exhibit when they lose their normal afferent input. When this hypersensitivity

arises in pathways that would normally contribute to pain sensation, the result is likely to be neuropathic pain perceived as arising from the denervated region. This pain is thus paradoxical in that the region where the person “feels” the pain is at least partially anesthetic, so for example, a pinprick in the painful region would not be perceived.

8.4. Sympathetic Efferent Mechanisms

It has been known since the past century that pain can be dependent on sympathetic activity in the painful area. This pain has been referred to as *reflex sympathetic dystrophy* or *causalgia* but is now termed *complex regional pain syndrome* (type I or II). Whatever you choose to call it, this syndrome of intense burning pain and allodynia usually develops after a traumatic lesion to peripheral nerve or deep tissue (muscle or bone). There are usually autonomic disturbances at some point in the course of the syndrome, and an important distinguishing feature is that pain can often be relieved by block of the sympathetic innervation to the painful area.

Because pain in complex regional pain syndromes is generally eliminated when activity in large-diameter afferents is blocked, it is thought that primary afferent nociceptors somehow become abnormally sensitive to norepinephrine released from sympathetic efferents. This promotes a continuous low level of activity in these neurons, which in turn sensitizes circuitry in the dorsal horn and supraspinal sites. Because of this central sensitization, input from large-diameter mechanoreceptive afferents is now sufficient to induce pain (Fig. 10).

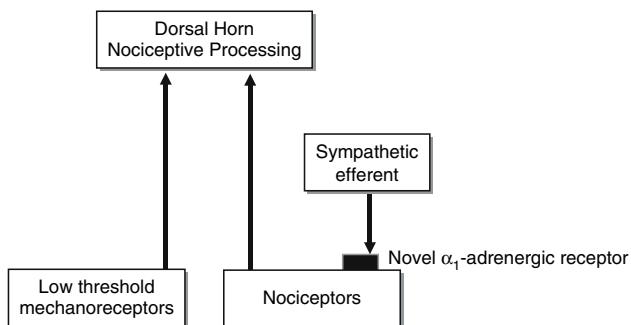


Fig. 10. Sympathetic mechanism of neuropathic pain. Initial injury causes nociceptors to develop a novel adrenoreceptor. Activity in sympathetic efferents thus evoked a continuing low level of activity in nociceptors, which in turn sensitized dorsal horn processing circuits. Because of this central sensitization, input from low-threshold mechanoreceptors now produces pain.

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Physical Trauma to Nerves

Gregory Cooper, Gerald Eichhorn and Robert Rodnitzky

Nerve injury can occur as a result of major acute trauma, such as a gunshot wound, or develop more slowly as a result of milder but continuous trauma. The degree of neurologic deficit and the prospect for recovery depend on several factors. Typically, acute injuries resulting in anatomic disruption of the nerve cause more severe and lasting disability than do slowly developing injuries that leave the nerve in anatomic continuity.

NERVE SEVERANCE RESULTS IN A PREDICTABLE SEQUENCE OF CHANGES IN THE NERVE AND MUSCLE

If nerve continuity is disrupted as a result of trauma, the nerve segment distal to the injury undergoes Wallerian degeneration. This process involves disintegration of axoplasm and axolemma and ultimately results in the breakdown and phagocytosis of myelin, taking several weeks to complete. Severe degeneration of the nerve implies that any recovery of function will require regeneration of nerve fibers from the intact nerve stump to the appropriate muscle or sensory organ.

After about 2 weeks, significant changes appear in the denervated muscle. Acetylcholine receptors begin to proliferate at locations on the muscle fibers outside the end-plate region, where they are usually concentrated. At the same time, spontaneous contractions of muscle fibers, known as fibrillations, appear. Fibrillations can be detected using electromyography, a common clinical electrodiagnostic technique in which the electrical potentials generated by muscle fibers can be measured through a small intramuscular needle electrode. Detection of fibrillation potentials suggests that the muscle is denervated. This finding

can be considered indirect evidence that the nerve fiber innervating the muscle has undergone axonal disruption.

Fibrillations, which can only be detected by electromyographic techniques, should not be confused with fasciculations, which are gross muscle twitchings under the skin that are clearly visible to the naked eye. Unlike fibrillations, which strongly suggest muscle denervations, fasciculations can be seen in both normal and denervated muscles.

NERVE-CONDUCTION TESTING IS USEFUL IN DETERMINING THE EXTENT AND LOCATION OF NERVE DAMAGE

Electrical stimulation of a motor nerve segment distal to the point of injury can help determine whether the injury has disrupted the nerve's anatomic continuity. Within 8 days after severance of a nerve, stimulation of the distal segment no longer produces a response in the muscle it innervates. However, if the damage to the nerve has only resulted in a physiologic block of impulse conduction (e.g., neurapraxia) but not anatomic discontinuity, a muscle response occurs, implying intact conduction in the nerve segment beyond the point of injury.

Nerve stimulation can also help to determine whether an injury to a sensory nerve is preganglionic or postganglionic. In preganglionic injury, the distal nerve fibers are still in continuity with the nerve ganglion, or cell body, and therefore viable. Stimulation of the nerve produces propagated impulses that can be recorded over a distant portion of the nerve, proving its viability. In a postganglionic lesion, the distal segment of the nerve, after being disconnected from the

nerve-cell body, degenerates and loses its ability to be stimulated and conduct an impulse.

CHRONIC COMPRESSION AND ENTRAPMENT ARE AMONG THE MOST COMMON FORMS OF NERVE INJURY

Compression, constriction, or stretching of nerves is common at certain anatomic sites that are vulnerable to these mechanical forces. A nerve may be susceptible to compression because of its superficial location. An example is the peroneal nerve below the knee, where it crosses over the lateral border of the top, or head, of the fibula just under the skin. Because of this superficial location, there is no protective layer of muscle or fat. The nerve is often damaged when one leg is crossed over the other and the fibular head comes to rest on the opposite knee. The ensuing peroneal-nerve palsy results in a pattern of muscle weakness causing footdrop.

Nerve compression resulting from passage through a confining space is exemplified by carpal tunnel syndrome. At the wrist, the median nerve passes under a thick, fibrous ligament and can be chronically compressed, especially during repetitive flexion of the wrist. This syndrome, unlike peroneal-nerve palsy, typically develops over a period of months as a result of less severe but more prolonged trauma. In severe forms of carpal tunnel syndrome, neurologic symptoms develop, including weakness of the muscles controlling the thumb and tingling of the thumb and the next two digits.

Stretching is a form of trauma that often affects the ulnar nerve. This nerve courses over the elbow and can be stretched when the elbow is flexed. With repeated stretching, an ulnar nerve palsy develops, producing symptoms that include weakness of the intrinsic hand muscles and numbness of the fourth and fifth digits of the hand. The susceptibility of the

ulnar nerve to compression at the elbow is known to anyone who has struck his or her “funny bone.”

RECOVERY AFTER NERVE TRAUMA MAY DEPEND ON NERVE REGENERATION

After Wallerian degeneration, nerve regeneration may occur, especially if the surrounding connective tissue elements of the nerve sheath are intact and guide the regenerating fibers in the proper direction. Even under these favorable circumstances, recovery may be delayed, because nerve regeneration proceeds at the rate of only about 1 mm per day. The forward progress of the regenerating nerve tip can be monitored by using the Tinel sign. Because the advancing edge of a regenerating nerve fiber is unmyelinated, it is unusually sensitive to minimal mechanical stimuli. By lightly tapping through the skin along the course of a regenerating nerve, the physician can identify the location of the advancing nerve tip. The tap produces a tingling sensation in the part of the body toward which the regenerating sensory nerve is growing.

One of the pitfalls of peripheral-nerve regeneration is that fibers, especially those with motor and autonomic functions, may become misdirected to a different muscle or gland than was originally innervated. This is known as aberrant regeneration and may be seen after recovery from Bell's palsy, in which the facial nerve is damaged. The misdirection of the regenerating facial nerve intended for the periorbital muscles grows instead to perioral muscles. An attempted eye blink then produces a simultaneous twitch of the side of the mouth. This dual action, caused by aberrant regeneration, is called synkinetic movement.

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Peripheral Neuropathy

Gregory Cooper, Gerald Eichhorn
and Robert L. Rodnitzky

The peripheral nerves are susceptible to toxic, metabolic, traumatic, and neoplastic damage. Regardless of the cause, the damage is known as neuropathy. Circumstances can alter the nature of the damage to the nerve. In some neuropathies, the axon is the primary focus of involvement. In some, the myelin sheath is largely involved, and in others, the axon and sheath are equally affected. The anatomic distribution of nerve involvement also varies. In some neuropathies, the most proximal portion of the nerve is involved, but in others, the most distal segment is primarily affected. Involvement of a single nerve is referred to as *mononeuropathy*, and the term *polyneuropathy* is used when nerves throughout the body are diffusely affected.

There are too many causes of peripheral neuropathy to list here, but among the most common are diabetes, kidney failure, chronic alcohol use and its associated nutritional deficiencies, autoimmune diseases, and trauma.

AXONAL DEGENERATION CAN TAKE SEVERAL FORMS

Several processes can result in axonal degeneration. Wallerian degeneration is common after severe nerve trauma. After any insult that interrupts the axon, the segment distal to the lesion undergoes progressive degeneration over the next several days. The myelin components of the distal-nerve segment degenerate, and fragments of the axon and myelin sheath are ultimately cleared by macrophages.

Many neuropathies that primarily affect the axon, especially those of toxic origin, are characterized by

degeneration of the distal portion of the axon through a process known as the dying-back phenomenon. This process usually involves the longest nerves in the body and is typically manifested first in the nerves innervating the feet and hands. This explains why sensory symptoms in many axonal neuropathies initially appear in the toes and feet or in the fingertips and hands. While many different conditions can lead to an axonal neuropathy, this dying-back phenomenon is probably largely related to the failure of axonal transport to support sufficiently the portions of the nerve that are most distant from the cell body.

Neuronopathy refers to a destructive process primarily involving the nerve-cell body, producing degeneration and loss of neurofilaments within the axon, which reduces the caliber of the axon. A sensory neuronopathy, manifesting as diffuse painful dysesthesias and sensory loss, has been associated with underlying malignancy, most often small cell lung cancer, and the presence of anti-Hu antibodies. This autoimmune sensory neuronopathy affects the dorsal-root ganglia, with symptoms commonly preceding the identification of the underlying cancer.

DEMYELINATION OF PERIPHERAL NERVES MAY BE PRIMARY OR SECONDARY

Segmental demyelination refers to the breakdown of the myelin sheath in the nerve segment between two nodes of Ranvier. These abnormal segments may be scattered along the length of the nerve. Because of the importance of the myelin sheath in facilitating rapid impulse conduction, segmental demyelination markedly slows nerve conduction and, in extreme

forms, produces total conduction block. These conduction abnormalities can be easily demonstrated in the clinical electrophysiology laboratory using the technique of nerve-conduction velocity testing. The term *secondary demyelination* is used to describe the myelin breakdown that occurs as the result of an antecedent primary axonal insult.

THE TYPICAL SYMPTOMS OF PERIPHERAL NEUROPATHY INCLUDE POSITIVE AND NEGATIVE PHENOMENA

The most common negative signs of peripheral-nerve dysfunction are the loss of strength and sensation. In axonal neuropathies, these signs begin in the feet and progress proximally, correlating with the predominance of pathology in the distal portion of long peripheral nerves. Even in demyelinating neuropathies, a similar distal pattern of sensory and motor loss is often observed, because longer nerve fibers are more likely than shorter ones to contain randomly distributed demyelinated foci. In addition to muscle weakness, involved muscles may demonstrate atrophy and fasciculations, which are signs of denervation.

Any sensory modality can be lost in a peripheral neuropathy. In conditions that primarily affect small fibers, pain and temperature sensation are preferentially lost, and in neuropathies involving large, myelinated fibers, there may a greater loss of vibratory and proprioceptive sensation.

One of the most prominent negative signs of peripheral-nerve disease is the loss of muscle-stretch reflexes. Reduction or loss of stretch reflexes is one of the most sensitive signs of peripheral nerve disease. There are several mechanisms by which these reflexes can be affected. In neuropathies involving large, rapidly conducting fibers, Ia afferent fibers from the muscle spindle may be directly involved, interrupting the afferent arc of the reflex. In neuropathies involving smaller fibers, the gamma efferent fibers to the spindle may be the locus at which the reflex is affected. Although large- and small-fiber neuropathies can produce areflexia, it is more common in cases of large-fiber involvement.

Involvement of autonomic fibers can produce symptoms such as a loss of sweating, abnormal heart rhythms, or impaired control of blood pressure. In most neuropathies, autonomic dysfunction is not prominent, but there can be significant autonomic symptoms in conditions resulting in acute demyelination or those primarily affecting small myelinated and

unmyelinated fibers. An example of the former is Guillain-Barré syndrome, and an example of the latter is the neuropathy of diabetes.

Sensory ataxia is another negative feature of peripheral neuropathy. It results from the severe loss of position sense in the feet. Involvement of large, myelinated fibers conveying proprioceptive sense prevents the patient from appreciating the exact position of the feet, resulting in an unsteady gait.

The most prominent positive symptoms of neuropathies are in the sensory realm. The term *paresthesia* is used to describe uncomfortable sensory perceptions occurring spontaneously without an apparent stimulus. These sensations are variously described as burning, tightness, tingling, or pins and needles. The term *dysesthesia* refers to an unusual or distorted sensation evoked by a stimulus such as a simple touch. Hyperalgesia is exaggerated pain perception that occurs in response to a stimulus that would ordinarily be painless. Spontaneously painful sensations are slightly more common in neuropathies involving small-diameter fibers.

Positive phenomena related to motor-nerve involvement include fasciculations and muscle cramps. A fasciculation is the spontaneous contraction of a denervated motor unit, which is a group of muscle fibers previously innervated by a single motor-nerve fiber. Fasciculations appear as flickering movements of muscles that can be seen through the skin. Normal muscles may occasionally exhibit fasciculations under conditions such as extreme fatigue.

NERVE-CONDUCTION TESTING IS USEFUL IN ANALYZING PERIPHERAL NEUROPATHIES

Nerve conduction velocity can be calculated by stimulating a nerve trunk at one point along its course and determining the time of arrival of the impulse at a measured distance along the nerve. This is a relatively painless procedure that requires a small electrical stimulus to be applied to the nerve through the skin. This technique can differentiate axonal from demyelinating neuropathies. Because an intact myelin sheath is critical for rapid impulse propagation, the nerve-conduction velocity is markedly diminished in demyelinating neuropathies. Conduction block, another electrophysiologic feature typical of demyelinating neuropathies, can also be demonstrated by this technique. By contrast, in axonal neuropathies, conduction velocity is only minimally slowed, but the amplitude of the evoked response in the nerve or in a muscle innervated by the nerve is clearly diminished.

The decreased amplitude results from the loss of conduction axons within the nerve.

In demyelinating neuropathies, recovery can occur through remyelination. However, even after recovery, nerve-conduction velocity may remain slow, because the nodes of Ranvier in re-myelinated segments may be closer together, resulting in less efficient saltatory conduction.

PERIPHERAL NEUROPATHY MAY DEVELOP ACUTELY OR CHRONICALLY

Most neuropathies caused by metabolic, degenerative, or heritable abnormalities develop over a period of several months or years. Chronic evolution is seen in the neuropathies associated with diabetes, kidney failure, or lead exposure, and this pattern is typical of most familial neuropathies. A smaller subset of neuropathies develop more rapidly.

The Guillain-Barré syndrome is an example of a neuropathy with a subacute onset. In this condition, autoimmune inflammatory demyelination of peripheral nerves occurs over a period of several days to 2 weeks. Within this period, the affected person may revert from total normality to a profound state of weakness, immobility, and respiratory insufficiency.

The onset of traumatic nerve disorders can be acute or chronic. An example of acute traumatic neuropathy is the sudden development of footdrop caused by acute compression of the peroneal nerve as it crosses the outside of the knee. This condition is often caused by crossing one leg over the other. A more slowly developing traumatic nerve disorder is carpal tunnel syndrome. It is caused by chronic or repetitive compression of the median nerve as it courses under a tight ligament at the wrist. Dysfunction of the nerve results in sensory loss and paresthesias involving the first three or four digits of the hand and weakness of the muscles controlling the thumb. Footdrop caused by peroneal-nerve compression

often recovers spontaneously if further trauma is avoided, but in carpal tunnel syndrome, trauma frequently continues, and the condition often requires treatment consisting of surgical release of the compressing ligament.

THERAPY FOR NEUROPATHIES CAN BE DIRECTED AT THE SYMPTOMS AND THE CAUSE

For most metabolic or toxic neuropathies, medical treatment is focused on correcting the underlying metabolic abnormality or reducing the amount of the toxic substance within the body. For example, in patients with kidney failure, renal dialysis is an extremely effective means of improving the associated neuropathy. In a toxic neuropathy such as that caused by lead intoxication, chelating agents, which promote the excretion of lead, are useful. Neuropathies that have an autoimmune basis are treated with immunosuppressive therapies. For patients with Guillain-Barré syndrome, plasma exchange and intravenous immunoglobulin (IVIg), both immunomodulatory treatments, have proved to be beneficial.

The treatment of the symptoms, as opposed to the cause of neuropathy, is largely confined to attempts at reducing pain and uncomfortable paresthesias. Anticonvulsant medications and tricyclic antidepressant drugs have been successfully used to reduce neuropathic pain. In neuropathies in which pain is localized to a discrete area such as the foot, capsaicin can be applied to the skin. This agent works by locally depleting substance P, a neuropeptide involved in the transmission of pain impulses.

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***J. Fielding Heitmancik, Edmond J. FitzGibbon
and Rafael C. Caruso***

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1. INTRODUCTION

Components of the visual system include the optical components of the eye (cornea, aqueous humor, lens, and vitreous body), retina, optic nerves, optic tracts, optic radiations, visual cortex, and a variety of nuclei.

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Each of these structures plays an important role in receiving and interpreting visual signals.

The optical components of the eye focus light on the retina, which transduces the light signal into neural signals. It also performs some initial processing before passing the neural signals through the optic nerves and tracts to central structures that perform more elaborate processing and integrate their information

with that of the other senses. Finally the oculomotor system, which serves as the efferent limb of the visual system, maintains the stability of eye position and directs eye movements toward objects of particular interest. This chapter considers the visual process and the anatomic components that carry it out, as well as the critical periods during which intercellular communication by synaptic transmission alters the fate of connections in the primary visual pathway from the retina to the visual cortex. In keeping with the philosophy of this volume, general principles are emphasized instead of experimental results. Students who wish to learn more about the experimental bases of these principles are directed to the Selected Readings provided at the end of this chapter.

2. REFRACTION

Initially, light passes through the cornea, aqueous humor, lens, and vitreous body (Fig. 1). Light travels through each of these relatively dense materials with a velocity that is inversely proportional to its density.

The refractive index of the material is defined as the ratio of the velocity of light in a vacuum to the velocity in that substance. When a light wave strikes the curved surface of the cornea at an angle, the waveform that enters the cornea first is slowed relative to that which travels a longer distance through the air. In this process, the path of a light ray is bent, a phenomenon known as refraction. If the components of the anterior segment of the eye—especially the cornea and lens—are shaped correctly, the light rays that emanate from a single point are focused onto a single point on the retina. The image of an object is projected onto the retina in an inverted fashion. Thus, the inferior part of the visual field is projected onto the superior part of the retina, the nasal visual fields are projected onto the temporal part of the retina, and the temporal visual fields are projected onto the nasal retina. Because human eyes are situated frontally, a significant fraction of each visual hemifield is viewed by both retinas and is known as the binocular visual field.

The refractive power of the eye resides primarily in four surfaces: the anterior and posterior surfaces of

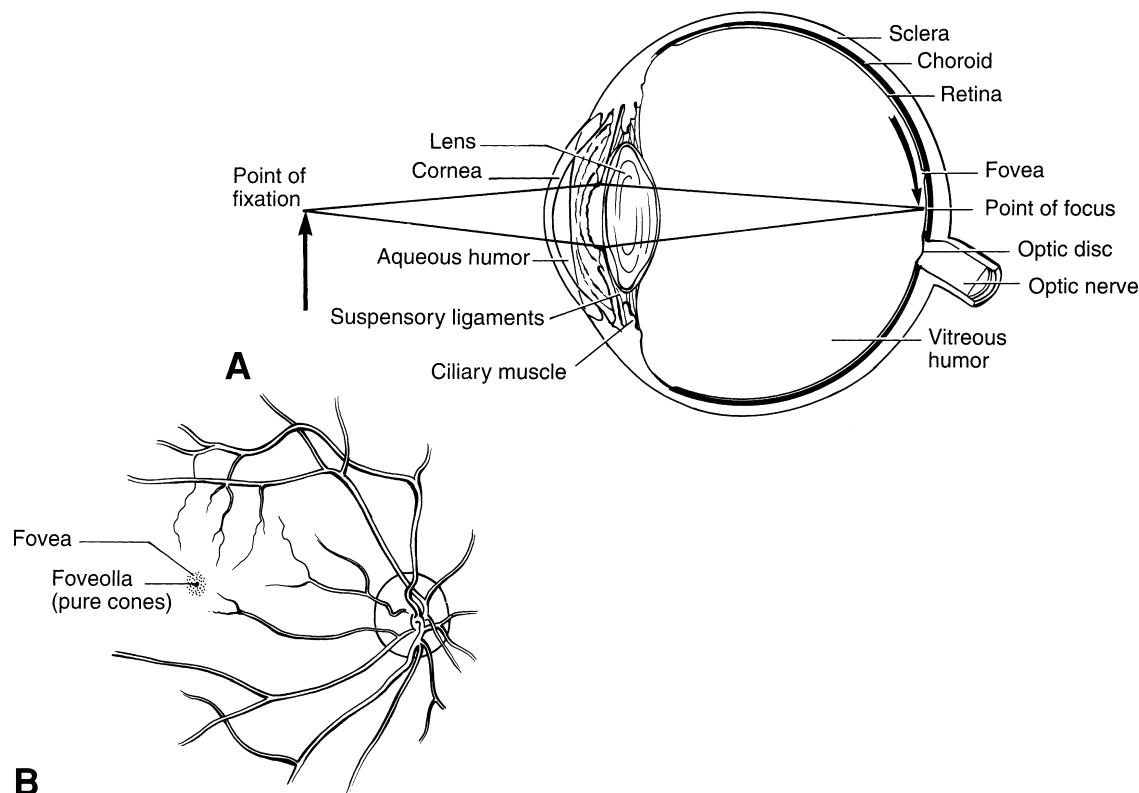


Fig. 1. (A) Overview of the eye and refraction. The refraction of light rays from the fixation point at the tip of the arrow to the focal point on the retina represents the summed effects of the anterior and posterior surfaces of the cornea and lens. The image of the arrow is projected in an inverted orientation on the retina. (B) View of the central retina, including the macular region and fovea on the left. The optic disk with emerging vessels, representing the optic nerve head, is shown on the right. It has no rods or cones and is optically insensitive.

the cornea and the anterior and posterior surfaces of the lens. Because the amount of refraction depends on the change in the refractive index between two substances, most refraction occurs at the anterior surface of the cornea, which is adjacent to air. When the eye is focused on a distant object, approximately one-third of the refractive power of the eye results from the lens. The degree of lens curvature depends on the contraction of the circular ciliary muscle. Contraction of the ciliary muscle decreases its diameter, allowing the lens to assume a more spherical shape. This change in the shape of the lens allows the eye to shift its focus from a distant point to a near point and is called accommodation.

A variety of refractive errors can occur. Hyperopia (e.g., farsightedness) is usually caused by a globe that is shortened anteroposteriorly or a lens system that is too weak and focuses light on a point behind the retina. Myopia (e.g., nearsightedness) usually results from an elongated anteroposterior diameter, which causes light to be focused anteriorly to the retina (e.g., on a point in the vitreous body). Astigmatism, a more complex refractive error, results from irregular curvature of the lens or, more commonly, of the cornea. This creates an optical element with stronger curvature in one meridian than in another, which focuses a point source of light into a line rather than into a point. These refractive errors can usually be corrected with appropriate lenses or, more recently, with surgery. Lens opacities (cataracts)

may occur and, if they are sufficiently severe, may require surgery for correction.

After light has been focused on the retina, this structure effects the transformation from a light signal into a neural signal. The retina and the higher neuronal centers interpret the information transduced by the retina. Despite intensive research, the molecular events underlying the transformation of light energy into neural information are still being elucidated.

3. THE RETINA

3.1. The Retina Consists of the Retinal Pigment Epithelium and the Neural Retina Containing Photoreceptors and Neuronal Processing Cells

The retina and its component cells have been described in great detail for vertebrate and invertebrate species. This discussion focuses on vertebrate vision, although much of the progress in understanding visual processes has come from studies of invertebrate models, which continue to be a valuable resource.

The structure of the vertebrate retina is shown schematically in Fig. 2. Many cell types contribute to the adult structure. The basic processes underlying retinal development are beginning to be understood but are outside the range of this discussion. It is sufficient for our purposes to mention that the retina consists of two structural and functional components: the retinal

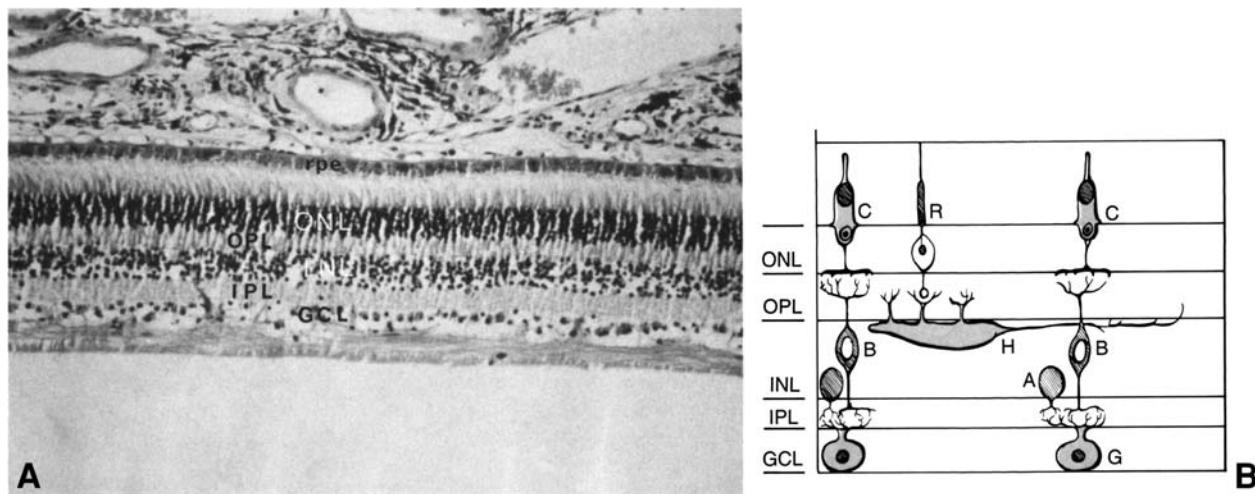


Fig. 2. The vertebrate retina. (A) Light microscopy section of a mouse retina. (B) Schematic of the cells in the retina. ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion-cell layer; R, rod cell; C, cone cell; B, bipolar cell; H, horizontal cell; A, amacrine cell; G, ganglion cell; RPE, retinal pigmented epithelium. (Hematoxylin & eosin stain; mouse retina at original magnification x200; image courtesy of Dr. Chi Chao Chan, National Institutes of Health, Bethesda, MD.)

pigment epithelium (RPE; the nonneural component) and the closely associated but physically distinct neural (i.e., sensory component) retina.

Cells of the retinal pigment epithelium contain melanin granules, which prevent light that is passed through the retina from being reflected by the sclera and degrading vision—a problem in disorders such as in albinism. The RPE cells also assist the photoreceptors with resynthesis of visual pigments and phagocytosis of shed outer-segment tips. Because this requires the outer segments containing these pigments to be closely approximated to the retinal-pigment epithelial layer, the neural processing networks of the retina are the anterior-most structures, and light must pass through them before stimulating the photoreceptor cells. The neural retina can be further divided into three nuclear layers separated by two plexiform (synaptic) layers. These layers are composed of six neuronal-cell types and the non-neuronal glial (Müller) cell. The interactions of these cell types are described later in this chapter.

Phototransduction, the biochemical process of transforming light to electrical energy, occurs in the *photoreceptor cells*. The highly specialized photoreceptor cells can be divided into rods or cones, depending on their morphology and function. The most notable feature of these cells is the outer segment, which consists of a series of stacked membranous disks, from which the cells derive their names. Rhodopsin, which absorbs light energy and initiates the transduction cascade, is located in the disks of the rod cells (Fig. 3). Rod cells are found at greater density in the near periphery of the retina and contain rhodopsin. Rods are able to detect light under dim illumination and are therefore important in night vision. The central retina, known as the macula, is densely populated with cone cells that mediate vision under strong (e.g., daylight) illumination.

Each cone contains one of three different opsins that vary in their peak wavelength absorption. Color vision relies on the comparison of the different degrees of stimulation of the three cone types by a colored stimulus. Another unique feature of the central portion of the macula—the fovea—that improves central vision is the absence of retinal elements other than photoreceptors. Axons from more peripheral retinal areas arc around the macula to minimize absorption and scattering of light in this critical part of the retina.

The photoreceptor cells, whose cell bodies make up the outer nuclear layer, synapse in the outer

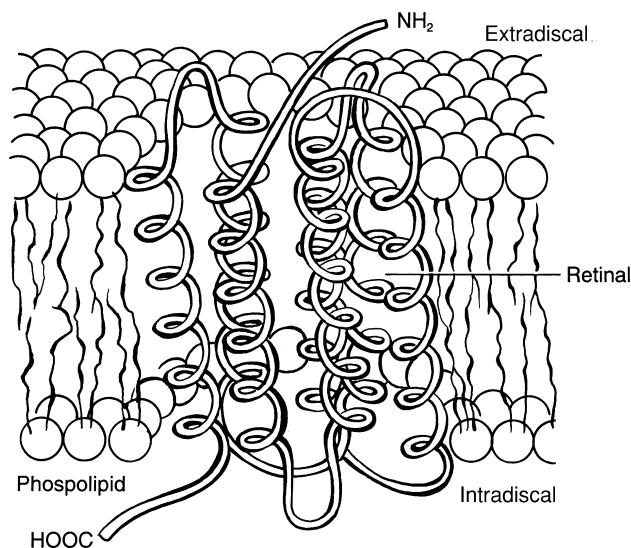


Fig. 3. The structure of human rhodopsin and its putative position in the membrane. A lysine in the seventh transmembrane domain is the site of the chromophore attachment (retinal).

plexiform layer of the retina with horizontal cells and bipolar cells. The bipolar cells synapse in the inner plexiform layer with amacrine and ganglion cells. The cell bodies of the amacrine, bipolar, horizontal, and interplexiform cells comprise the inner nuclear layer, and the cell bodies of the ganglion cells constitute the ganglion-cell layer. The ganglion-cell axons traverse the nerve fiber layer of the retina and collect in the optic nerve, which leads to the brain. The specific nature of these neural interactions is considered in the following sections.

4. PHOTOTRANSDUCTION

4.1. Continuously Graded Signals Are Generated in the Photoreceptors by Activation of Opsin-Chromophore Complexes

In the neural retina, the information contained in light absorbed by the photoreceptors is converted by them into neural signals in a process called *phototransduction*. Phototransduction is a model for understanding more general signal-transduction processes. For example, opsins show homology to a family of hormone receptors, including adrenergic receptors that activate adenylyl cyclase through G protein-coupled receptors (GPCRs). Figure 3 depicts the structure of the rhodopsin molecule in the disk membrane. Figure 4 shows a representation of the overall process of phototransduction. A photon of

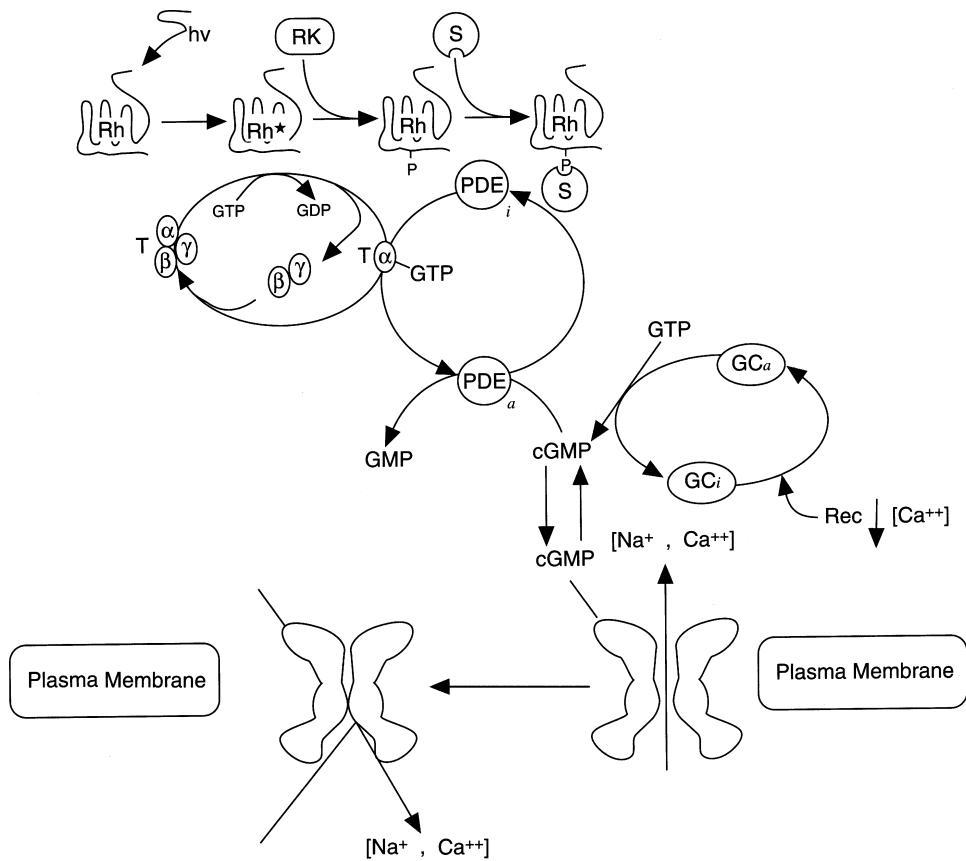


Fig. 4. Phototransduction. The light activation of rhodopsin ($\text{Rh} \rightarrow \text{Rh}^*$) activates transducin (T), the photoreceptor-specific G protein, by the exchange of GTP for GDP. The T α subunit activates the cGMP phosphodiesterase (PDE), cleaving cGMP and closing the ion channels, resulting in hyperpolarization and subsequent propagation of the electrical impulse. Two pathways involved in modulating the photoresponse are also shown. The first involves phosphorylation of rhodopsin by rhodopsin kinase (RK), with subsequent binding by S-antigen, also known as arrestin or the 48-kDa protein (S). The second pathway involves the regeneration of cGMP through stimulation of guanylate cyclase by recoverin (rec) under a reduced calcium level. Recoverin is also known as the 26-kDa protein.

light is absorbed by the opsin-chromophore complex (e.g., rhodopsin in rods, cone opsins in cones) in the outer segment of the cone cell or rod cell, changing the chromophore retinal from the 11-cis to the all-trans conformation. This conformational change results in the activation of transducin (e.g., photoreceptor-specific G protein) by the exchange of a bound guanosine diphosphate (GDP) for guanosine triphosphate (GTP). The activated transducin stimulates a cGMP phosphodiesterase (PDE), which then cleaves cyclic guanosine monophosphate (cGMP). Reduction of cGMP levels closes the cGMP-gated ion channels, causing intracellular hyperpolarization. This in turn results in closure of calcium channels and a subsequent decrease in glutamate release in the synaptic terminal of the photoreceptor (e.g., signal generation).

Rod cells have a low threshold of excitation and react readily to the low intensities of light required

for vision at twilight and at nighttime. The cones require a much higher intensity of stimulatory light and are important for fine vision and color discrimination. This is especially true in the posterior pole, where the high concentration of cones in the macula and fovea centralis are responsible for central vision.

Because the eye must provide sensitivity over a range of light intensities and wavelengths, the basic transduction process is modulated by several mechanisms. For example, it is known that phosphorylation of rhodopsin by rhodopsin kinase after light stimulation is required for effective quenching of the signal through the interaction of phospho-rhodopsin and arrestin (also known as S antigen). The phosphorylation or dephosphorylation of a PDE subunit is also important in modulating the light-induced response. Other mechanisms involved directly or indirectly in the *transduction cascade* include modulation by Ca^{2+} levels, which are

implicated in dark and light adaptation, regulation of cGMP concentration, and the dissociation and reassociation of the opsin protein moiety with its chromophore after light activation. Phosphorylation and dephosphorylation of several different components of the cascade probably also play a regulatory role.

Other biochemical cascades appear to be initiated by photoactivation of rhodopsin. For example, activation of retinal phospholipase C, which cleaves phosphatidylinositol bisphosphate to diacylglycerol and inositol triphosphate and phospholipase A2, which releases arachidonic acid, have been shown to be light-dependent. The role of these pathways and probably others in the normal physiology of the retina is unclear. It is likely that they are involved in some type of second-order modulation of the photo response or possibly they are involved in housekeeping-type functions, such as signaling the turnover (e.g., shedding) of disks. The identification of molecules involved in these processes is important for understanding the molecular basis of vision and for identifying the possible causes of retinal lesions.

4.2. Neural Transmission and Processing in the Retina

Photoreceptor cells transduce visual stimuli to other cells of the neural retina as continuously graded changes in membrane potential. The efferent retinal neurons (ganglion cells) send this information through the optic pathways as a series of all-or-none signals (e.g., action potentials) with enhanced color, motion, and contrast detection. The interaction of the neural cells of the retina was described previously and is shown in Fig. 2. The importance of retinal processing is seen in the response of an individual ganglion cell within its receptive field. The receptive field of a cell is the part of the retina in which stimulation of photoreceptors with light causes activation of that cell, as demonstrated by an increase (for "on" ganglion cells) or decrease (for "off" ganglion cells) in its firing rate. For ganglion cells, the receptive field is a roughly circular area of retina that corresponds with less than 1° of visual field at the fovea, which is the retinal area with tightly packed cones providing the finest visual discrimination, to 3° to 5° at the retinal periphery. For "on" ganglion cells, light that strikes the center of the receptive field is stimulatory, and light striking the periphery, known as the surround, is inhibitory. Conversely, for "off" ganglion cells, light that strikes the center is inhibitory. Both types of ganglion cells are maximally stimulated by large contrasts in the intensity of light striking the center and

periphery of their receptive fields, rather than an evenly spread illumination. It is a general principle that the visual system responds primarily to changes in the time, location, or intensity of visual input.

The importance of the concept of a receptive field is seen when retinal circuitry is examined in more detail. The photoreceptor cells send their input to the bipolar and horizontal cells. The horizontal cells appear to function as inhibitory neurons, using gamma-aminobutyric acid (GABA) as a neurotransmitter to perform negative-feedback control in the distal retina. They receive input from photoreceptors in the surround of a receptive field and pass an inhibitory signal on to the photoreceptors (negative feedback) and bipolar cells. This enhances contrast, emphasizing sharp edges and compensating for the blurring of the image caused by scattering of light by the various optical media of the eye.

Some bipolar cells are stimulatory (e.g., on), using glutamate to synapse to ganglion cells with their dendrites in the inner portions of the inner plexiform layer. Some are inhibitory (e.g., off), synapsing to ganglion cells with dendrites in the outer portion of the inner plexiform layer. The ganglion cells convert the graded light signal they have received from the bipolar cells into all-or-none signals (action potentials), typical of information-handling in the brain. They amplify visual signals and have on-center or off-center receptive fields. Some ganglion cells receive input from both types of bipolar cells, as do some amacrine cells. Although the interactions of amacrine cells are limited to the inner plexiform layer, they synapse with all the cell types found there.

Reflecting the various interactions, there appear to be different subsets of amacrine cells: inhibitory cells that use glycine as a neurotransmitter and excitatory cells that use acetylcholine. Some of these appear to be involved in light adaptation of visual sensitivity independently of mechanisms present in the photoreceptors. Amacrine cells also modulate the time course of a visual response. Some amacrine cells, like ganglion cells, generate action potentials, unlike photoreceptors, bipolar cells, and horizontal cells, which generate sustained and graded voltage potentials. The ganglion-cell layer consists of ganglion-cell bodies and a few amacrine cells. The ganglion cells send the final relay, turning the input they receive from bipolar and amacrine cells into action potentials that are then sent to the brain for further processing. It is impressive that more than half of all ganglion cells receive input solely from the foveal and parafoveal area, which makes up only about 5% of the

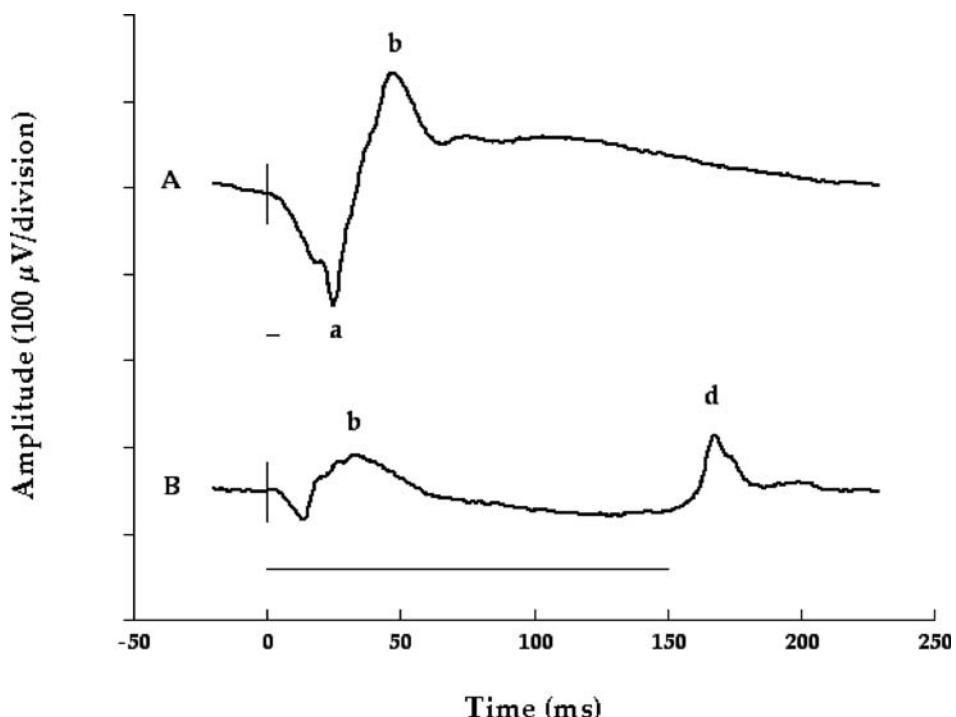


Fig. 5. Normal human electroretinogram (ERG). (A) ERG elicited by a brief (4 ms) flash on a dark-adapted retina. The a-wave (**a**) is generated primarily by hyperpolarization of photoreceptors; the b-wave (**b**) is generated primarily by depolarization of rod bipolar cells and cone on-bipolar cells. (B) ERG elicited by a longer (150 ms) flash on a light-adapted retina. The b-wave (**b**) reflects the depolarization of cone on-bipolar cells elicited by stimulus onset; the d-wave (**d**) reflects the depolarization of cone off-bipolar cells elicited by stimulus offset.

retinal surface. Thus, there is an extreme bias toward central (e.g., fine) vision rather than peripheral (coarser) visual perception.

The number of bipolar cells is much smaller than the number of photoreceptor cells, and there is an even further reduction in the relative number of ganglion cells. In humans, there may be as many as 140 million rods and cones but only 1 million ganglion cells. Each photoreceptor cell contacts a number of different neurons, and each neuron has several different synapses. The integrative abilities of the retinal neurons are probably indispensable for sorting out the signals.

Contrary to what might be expected, photoreceptor cells are actually depolarized in the dark and hyperpolarized in the light. In the on or dark state, glutamate is released by the photoreceptors at bipolar and horizontal cell synapses. The horizontal cells are also on in the dark as a result.

The on bipolar cells are inhibited by the photoreceptor cells' release of glutamate and are excited in the light, whereas the off bipolar cells are excited by glutamate and therefore are excited in the dark.

The electrical activity of the various retinal neurons can be measured by a noninvasive procedure

known as an electroretinogram (ERG). The ERG is similar to the electrocardiogram (ECG) and electroencephalogram (EEG) in that it assesses the activity of large numbers of cells by measuring the changing potential difference between a corneal and a reference electrode. Figure 5 depicts a highly idealized ERG tracing. The a-wave indicates photoreceptor activity, the b-wave indicates activity in the inner nuclear layer (especially bipolar and Müller cells), and the d-wave indicates the off response of the inner nuclear layer. Because different components of the ERG can be attributed to different cell types in the retina, it is clinically useful to identify possible retinal dysfunction and to narrow the diagnostic focus to specific cell types when looking for the source of visual difficulties.

5. THE OPTIC PATHWAYS

Axons of the ganglion cells collect in fine bundles that converge in a radiating pattern at the optic disk to form the optic nerve, connecting the eye to the brain. Because the optic disk contains no photoreceptors, it is insensitive to light, causing a small blind spot that is noticeable only on monocular vision. On,

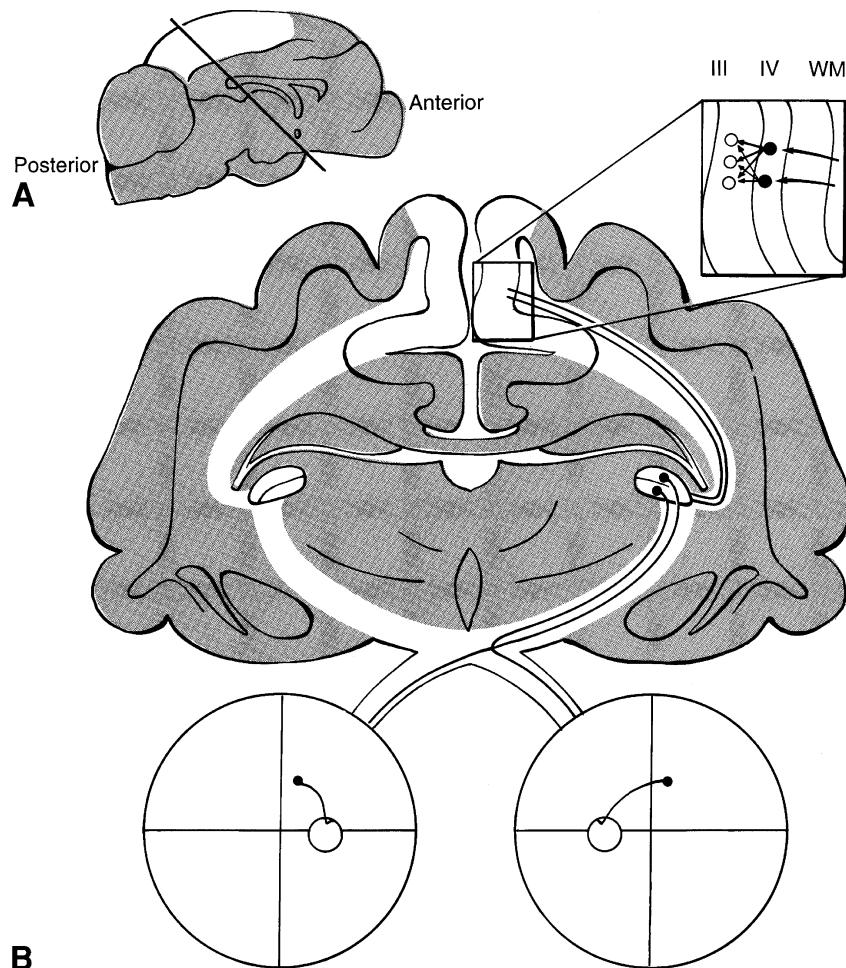


Fig. 6. Organization of the mammalian retinogeniculocortical projection. (A) Midsagittal view of a cat brain showing the location of the primary visual cortex (e.g., striate cortex or area 17). The line indicates a plane of section (illustrated in B) that reveals all the components of the ascending visual pathway. (B) The temporal retina of the left eye and the nasal retina of the right eye project axons through the optic nerve and optic tract to the LGN of the left dorsal thalamus. Inputs from the two eyes remain segregated in separate laminae at the level of this synaptic relay. The lateral geniculate cells project on to striate cortex through the optic radiations. These axons terminate mainly in layer IV, where inputs subserving the two eyes continue to be segregated. (Inset) The first site of major convergence of inputs from the two eyes is in the projection of layer IV cells onto cells in layer III. WM, white matter. (Modified from Baer MF, Cooper LN. Molecular mechanisms of synaptic modification in the visual cortex: interaction of theory and experiment. In Gluck M, Rumelhart D (eds.). *Neuroscience and Connectionist Theory*. Hillsdale, NJ: Lawrence Erlbaum Associates, 1990:65.)

off, color, and movement-related signals are passed to the brain through a set of closely approximated parallel channels. The spatial relations of the visual field are maintained in the fibers that make up the optic nerves and optic radiations to the visual cortex, with fibers from the left half of each retina running on the left part of the optic nerves. After passing through the optic foramina, the right and left optic nerves converge at the optic chiasm. At this point, nerve fibers originating in the nasal halves of the retina cross to the opposite side, and those from the temporal retinal halves continue uncrossed. As a result of

this pattern, the entire right visual field (received by the left temporal and right nasal halves of the retinas) projects to the left hemisphere, and the left visual field (received by the right temporal and left nasal halves of the retinas) projects to the right hemisphere (Fig. 6). Beyond the optic chiasm, the optic fibers continue on to the thalamus as the optic tract.

Some fibers continue to the superior colliculus and the pretectal area of the midbrain, which actuate reflex responses of the eyes and body to visual stimuli and the pupillary light reflex, respectively. However, most of the optic tract fibers synapse on neurons in

the lateral geniculate nucleus (LGN), consistent with its role as the most important subcortical region for further visual processing. The spatial relations of the retina and visual field continue to be maintained in the lateral geniculate body, creating a retinotopic map of the visual field. Inputs from the two eyes (i.e., the nasal retina of the contralateral eye and the temporal retina of the ipsilateral eye) remain anatomically segregated in the LGN, with separate layers of LGN neurons receiving the inputs from each eye. The retinotopic maps of the binocular field from each eye are in register; with a small monocular segment representing the far periphery of the visual field of the contralateral eye that is also present (Fig. 7). Different classes of ganglion cells in each retina project fibers to three distinct cell layers in the LGN, giving a total of six layers in all.

Axons of the upper quadrants (receiving the lower visual fields) project to the medial laminae, and these cells project as the geniculostriate pathway or optic radiation to the cerebral cortex in the superior edge of the calcarine sulcus. Axons of the lower quadrants (receiving the upper visual fields) project to the lateral half of the lateral geniculate body, and these cells then project to the inferior lip of the calcarine sulcus. The cortical area that receives LGN input is known as the primary visual cortex (V1), striate cortex, or Brodmann's area 17.

The main target of LGN neurons is a band of cells in the middle of the cortex, known as layer IV. The input from the different LGN layers that subserve the two eyes are not intermingled in layer IV.

This was elegantly shown by Hubel and Weisel by transmission of radioactive proline injected into

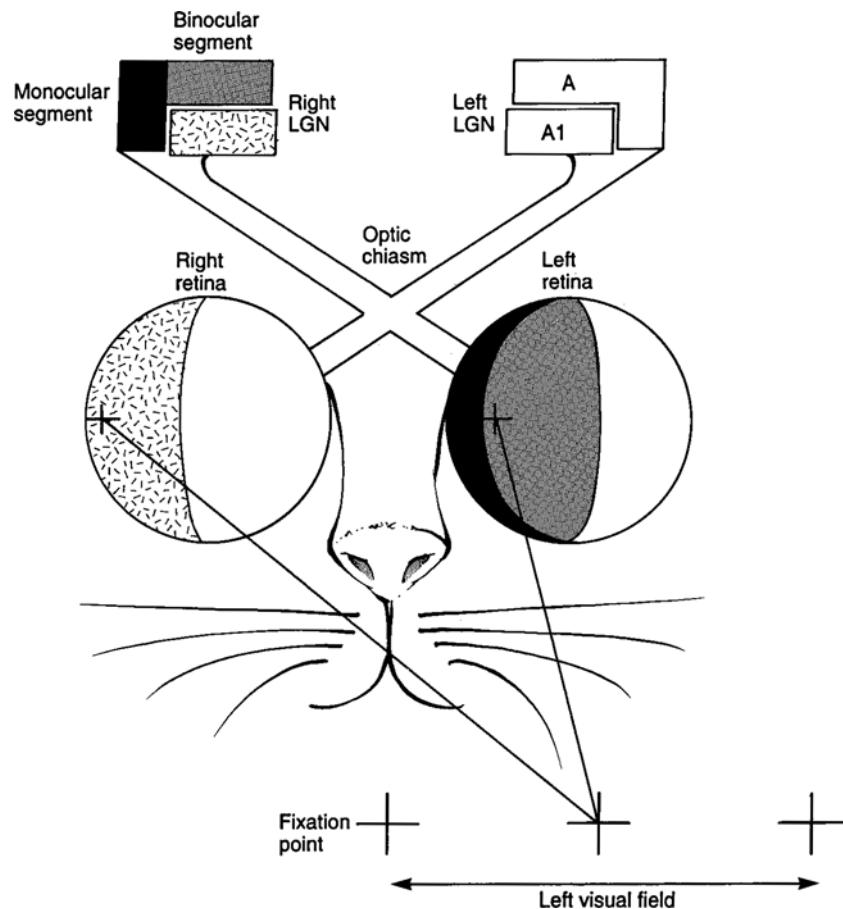


Fig. 7. The general organization of the lateral geniculate nucleus (LGN). Each eye projects to a separate cell layer in the lateral geniculate. In the cat, the principal layers are called A and A1. Layer A receives input from the nasal half of the contralateral retina, and layer A1 receives input from the temporal half of the ipsilateral retina. The retinotopic maps in the two layers of the lateral geniculate occur in perfect register, except for a lateral region of layer A called the monocular segment. Cells in the monocular segment are activated by visual stimuli in the far periphery that fall outside the binocular visual field and are viewed only by the contralateral eye.

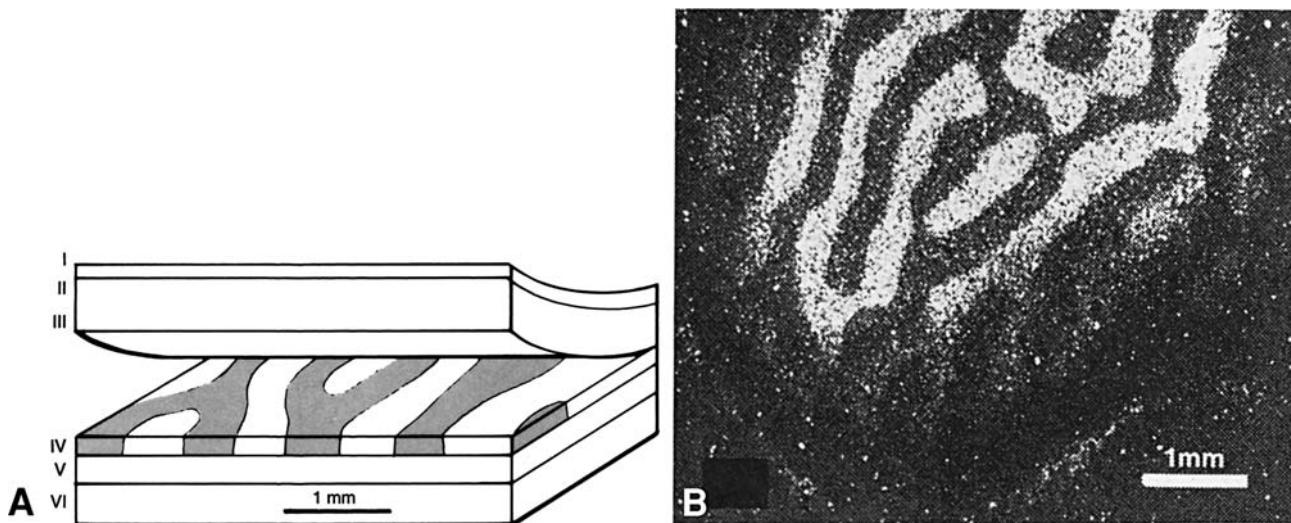


Fig. 8. (A) The organization of ocular-dominance columns in layer IV of the striate cortex of macaque monkeys. The distribution of geniculate afferents that subserve one eye is darkly shaded. In cross section, these eye-specific zones appear as columns of approximately 0.5-mm width in layer IV. When the superficial layers are peeled back, allowing a view of the ocular-dominance columns in layer IV from above, these zones take on the appearance of zebra stripes. (B) Dark-field autoradiograph of a histologic section of layer IV viewed from above. Two weeks before sacrifice, this monkey received an injection of ^{3}H -proline into one eye. In the autoradiograph, the radioactive lateral geniculate terminals appear bright on a dark background. (From Wiesel TN. Postnatal development of the visual cortex and the influence of the environment. Nature 1982;299:583.)

one eye to eye-specific dominance columns in layer IV of the striate cortex (Fig. 8). The proline was transmitted down the optic nerve into the LGN, where it spilled over through synaptic junctions to LGN neurons and was further transmitted down these neurons to layer IV. The roughly 1 million ganglion cells in each eye feed information to a similar number of lateral geniculate neurons with little transformation in the receptive fields by this synaptic relay. Similarly, the layer IV neurons that receive synaptic input from the LGN are characterized by small, circular, monocular receptive fields. Cells of this layer feed information to cells in the more superficial cortical layers, especially layer III, in which axons from different ocular-dominance columns project onto the same cells.

Thus, most neurons in layer III are responsive to stimulation of both eyes, and the receptive fields mapped through the two eyes are matched to the same position in space. This convergence initiates the process of binocular vision, in which information from the two eyes is combined to form a single perception of visual space. Neurons from layer III then project to other cortical areas. The first significant elaboration of receptive fields occurs at the projection from layer IV to layer III. Layer III neurons have elongated receptive fields and are especially

responsive to elongated, high-contrast bars or edges with the same orientation as the long axis of the receptive field, known as *orientation selectivity*. Thus, most cortical neurons respond poorly or not at all to changes in diffuse illumination of the retina. As a result of both excitatory and inhibitory intracortical connections, cortical neurons respond best to contrast borders.

From the striate cortex, there are additional projections to a surprisingly large number of other (extra-striate) visual areas of the cortex. For example, it is known that areas V₁ and V₂ integrate most of the visual information regarding color, movement, and form, and that other areas play some role in fine-tuning that information. The geniculo-cortical pathway is the most important for the conscious experience of vision in mammals. People with lesions of the striate cortex claim to be completely or almost completely blind. However, they are also partially able to identify or locate some objects, indicating that the retinotectal pathway is able to supply some type of vision, albeit subconsciously. These pathways are not independent of each other, because there is also a corticotectal path that provides input from the cortex to the superior colliculi. In addition to these two pathways, there are several minor pathways that are not well-understood. These project to the

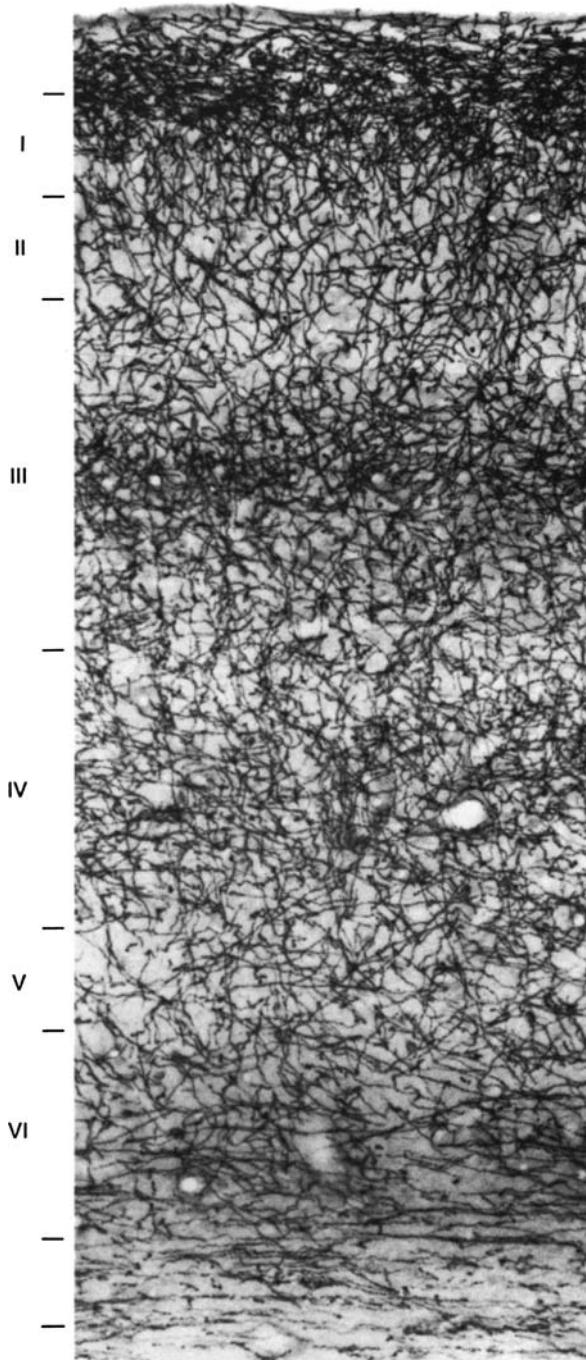


Fig. 9. Distribution of fibers that use the neurotransmitter acetylcholine in the cat striate cortex. Unlike the projection from the thalamus, this projection from the basal forebrain innervates all cortical layers. These cortical inputs modulate the activity patterns that arise from retinal stimulation and are believed to influence experience-dependent aspects of cortical development. (From Bear MF, Carnes KM, Ebner FF. An investigation of cholinergic circuitry in cat striate cortex using acetylcholinesterase histochemistry. *J Comp Neurol* 1985;234:411.)

ventral lateral geniculate (or pregeniculate) nuclei, tegmentum, and hypothalamus. This hypothalamic projection is probably involved in the synchronization of the circadian rhythm with the day/night cycle.

The cortex also receives input from the same cortical areas to which it projects, including the cortex of the opposite hemisphere through the corpus callosum. There are brain-stem inputs originating in the locus coeruleus that use epinephrine as a neurotransmitter and the Raphe nuclei using serotonin as a neurotransmitter. Unlike LGN inputs, the axons from the brain stem project to a variety of cortical layers, and their fibers ramify widely. A similar organization is seen in the projection from the nucleus basalis of Meynert in the forebrain, which uses acetylcholine as a neurotransmitter (Fig. 9). Based on their anatomy and the effects of applying their neurotransmitters to cortical neurons, these inputs appear to function in modulating visual processing according to behavioral state.

6. CRITICAL PERIODS IN VISUAL SYSTEM DEVELOPMENT

A *critical period* of development may be defined as a period of time in which intercellular communication alters a cell's fate. The concept is usually credited to the experimental embryologist Hans Spemann, who—working around the turn of the 20th century—showed that transplantation of a piece of early embryo from one location to another would often cause the “donor” tissue to take on the characteristics of the “host,” but only if transplantation had taken place during a well-defined period. After the transplanted tissue had been induced to change its developmental fate, the outcome could not be reversed. The intercellular communication that altered the phenotype of the transplanted cells was shown to be mediated by contact and by chemical signals.

The term took on new significance with respect to brain development as a result of the work of Konrad Lorenz in the mid-1930s. Lorenz was interested in the process by which graylag goslings come to be socially attached to their mother. He discovered that, in the absence of the mother, the social attachment could occur instead to a wide variety of moving objects, including Lorenz himself. Once imprinted on an object, the goslings followed it and behaved toward it as they normally would their mother. The term *imprinting* was used by Lorenz to suggest that this first visual image was somehow permanently etched

in the young bird's nervous system. Imprinting was also found to be limited to a finite time (e.g., the first 2 days after hatching), which Lorenz called the *critical period for social attachment*. Lorenz himself drew the analogy between this process of imprinting the external environment on the nervous system and the induction of tissue to change its developmental fate during critical periods of embryonic development.

This work had a tremendous impact in the field of developmental psychology. The terms *imprinting* and *critical period* conjure images (and generated heated debate) that changes in "behavioral phenotype" caused by early sensory experience were permanent and irreversible later in life, much like the determination of tissue phenotype during embryogenesis. Numerous studies extended the critical period concept to aspects of mammalian psychosocial development. The implication was that the fate of neurons and neural circuits in the brain depended on the experience of the animal during early postnatal life. It is not difficult to appreciate why research in this area took on political as well as scientific significance.

By necessity, the effects of experience on neuronal fate must be exercised by neural activity generated at sensory receptors and communicated by chemical synaptic transmission. The idea that synaptic activity can alter the fate of neuronal connectivity during central nervous system (CNS) development eventually received solid neurobiologic support from the study of mammalian visual system development, beginning with the experiments of Hubel and Wiesel that partly earned them the 1981 Nobel Prize in Medicine. They found, using anatomic and neurophysiologic methods, that visual experience or lack thereof was an important determinant of the state of connectivity in the central visual pathways and that this environmental influence was restricted to a finite period of early postnatal life. Because much work has been devoted to the analysis of experience-dependent plasticity of connections in this system, this is an excellent model system to illustrate the principles of critical periods in nervous-system development. Although the effects of synaptic transmission in the development of other systems (e.g., neuromuscular connections) and the effects of other types of intercellular communication (e.g., long-range hormonal signals) are not covered in detail here, the general principles of other critical periods are believed to be quite similar to those illustrated by visual-system development.

7. ADULT ORGANIZATION OF THE VISUAL PATHWAY FROM RETINA TO CORTEX

7.1. *The Anatomic and Physiologic Organization of the Visual Pathway Is Precise*

The detailed structure and function of the visual system is covered earlier in this chapter. Certain aspects are reviewed and emphasized here to provide a perspective on what must be accomplished during development to ensure proper wiring and function of this sensory system. The central visual pathway begins with the projection of ganglion-cell axons from the retina into the optic nerve. In general, the ganglion cells that "view" the right visual hemifield project to the left hemisphere of the brain. Conversely, the ganglion cells that are responsive to visual stimuli in the left visual hemifield project to the right hemisphere. Because human eyes are situated frontally in the head, a significant fraction of each visual hemifield is viewed by both retinas. This region of space is the binocular visual field. Each retina must project to both hemispheres; ganglion cells in the nasal retina (e.g., the half closer to the nose) project across the midline to the contralateral hemisphere, and ganglion cells in the temporal retina project axons into the hemisphere of the ipsilateral side.

8. ACTIVITY-INDEPENDENT DEVELOPMENT OF ORDER IN THE VISUAL SYSTEM

The preceding discussion indicates that there is considerable precision in the connections of the mature visual pathway. There are several examples:

1. Only ganglion-cell axons from nasal retinas cross at the chiasm.
2. The mixed population of axons in the optic tract is sorted out at the lateral geniculate nucleus by eye and by retinotopic position.
3. The LGN axons project to a specific layer of cells in the cortex, and within this layer, they segregate again according to retinotopic position and by eye.
4. Layer IV cells make connections with cells in other layers that are appropriate for binocular vision and are specialized to enable detection of contrast borders.

Before addressing the question of the extent to which the establishment of these highly specific connections depends on activity during critical periods of development, it is important to recognize that activity often precedes experience during CNS development. Action potentials and chemical synaptic

transmission occur in the visual pathway *in utero*, even before the development of the photoreceptors. Thus, the occurrence of a developmental process before visual input does not imply that it is independent of activity. One way to evaluate the dependence on activity is through the drug tetrodotoxin (TTX), which binds tightly to voltage-sensitive sodium channels and blocks action potentials. Thus, developmental processes that are insensitive to TTX are considered to occur independently of intercellular communication, and those that are sensitive are dependent on synaptic activity.

Development of long-range connections in the CNS can be categorized into three phases: pathway selection, target selection, and address selection. Examples of pathway selection include the decisions made by growing axons that originate in the nasal retina to cross the midline at the optic chiasm, and the decisions made by LGN axons to project to the cortex through the optic radiation rather than to the spinal cord via the cerebral peduncle. Examples of target selection include the decision made by optic-tract axons to form connections with the LGN and not another part of the thalamus, and the decision made by LGN axons to innervate cortical layer IV instead of layer V. Examples of address selection are the sorting of inputs by retinotopic location in the LGN and cortex, the segregation of axons that subserve the two eyes in the LGN and layer IV of the cortex, and the convergence of retinotopically matched inputs from the two eyes onto layer III neurons.

Experimental evidence has now shown that pathway and target selection occur entirely in the absence of neural activity. Many aspects of address selection, such as the establishment of crude retinotopy, are also activity-independent. This does not indicate that these aspects of development occur independently of intercellular communication, because virtually all phases of visual-pathway development critically depend on communication by cell-cell contact and by gradients of diffusible chemicals. The process by which specific connections are established by differential chemical attraction or repulsion is termed *chemoaffinity*.

The axons from the retina grow along the substrate provided by the extracellular matrix (ECM) of the ventral wall of the optic stalk. One glycoprotein in this matrix is laminin. The growing tips of the axons, called growth cones, express surface molecules called integrins that bind laminin, and this interaction promotes axonal elongation (Fig. 10). The ECM along the optic stalk forms a molecular highway on which

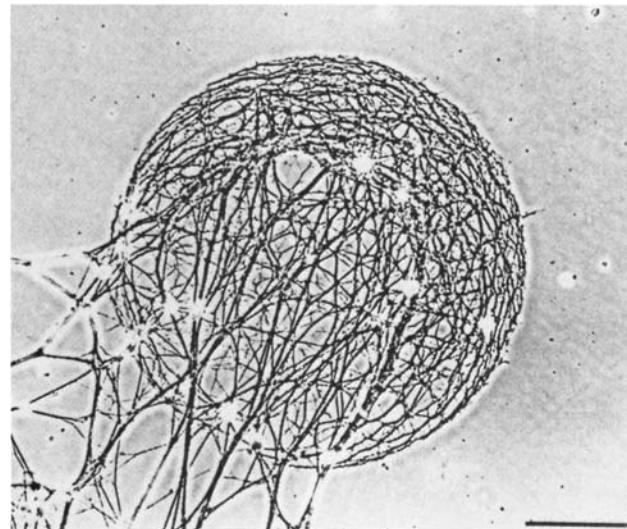


Fig. 10. Neurites that grow in a collagen-coated culture dish enter a region containing the extracellular matrix (ECM) protein laminin. On this preferred substrate, the neurites branch profusely but stay within the border of the circular laminin dot. The interplay of ECM and specific axon-surface molecules is believed to be crucial for pathway and target selection during visual-system development. (From Gunderson RW. Response of sensory neurites and growth cones to patterned substrata and fibronectin *in vitro*. Dev Biol 1987;121:423.)

retinal axons grow. The journey down this highway is aided by another mechanism that causes axons that are growing together to stick together, a process known as fasciculation. This stickiness is caused by the expression of specific cell-adhesion molecules on the surface of axonal membranes.

The ECM can be repulsive as well as attractive to growing axons, depending on the cell-surface receptors the axons express. Axons from the temporal retina grow toward the midline at the chiasm, but they encounter a signal there that causes them to veer sharply away. In contrast, nasal axons continue right across the midline and into the contralateral optic tract. These differences must be explained by differential expression of cell-surface molecules based on cell position in the retina. Such a nasal-temporal gradient in axon-surface markers is believed to be matched to complementary gradients on the surfaces of cells in target structures, and this match gives rise to retinotopy.

When axons reach their target, they often encounter a new extracellular environment that retards further growth. These environmental signals can be the absence of specific glycoproteins in the ECM, such as the absence of laminin. Axonal growth also can be inhibited by diffusible signals released from

target structures. In some systems, application of neurotransmitters can inhibit axonal growth, probably by raising calcium concentrations in the growth cone. There is evidence that diffusible factors can promote axon growth and guide that growth to distant targets in a process known as chemotropism. The number and nature of the chemical signals that guide activity-independent pathway formation are currently being investigated.

Considerable order can develop in the visual system solely under the influence of these molecular mechanisms. The role of activity appears to be reserved for the final refinement of the patterns of connectivity, according to functional criteria.

9. ACTIVITY-DEPENDENT DEVELOPMENT OF ORDER IN THE VISUAL SYSTEM

Much of the organization of the visual pathway is specified without any contribution from neural activity and synaptic transmission. The retinal and lateral geniculate axons navigate down the appropriate paths and terminate in retinotopic order in the appropriate target structure. The main features of visual-system organization that depend on retinal activity are the segregation of axons in the LGN and cortex according to the eye that drives activity in them and the establishment and maintenance of connections in the visual cortex that generate binocular and stimulus-selective receptive fields. The eye-specific segregation in the LGN occurs entirely before birth, but many refinements of cortical circuitry occur postnatally and are under the influence of the visual environment during infancy.

9.1. Segregation of Axons in the Lateral Geniculate Nucleus Is Influenced by Activity

The first axons to reach the LGN are usually those from the contralateral retina, and they disperse to occupy the entire nucleus. Somewhat later, the ipsilateral projection arrives and intermingles with the axons of the contralateral eye. Over the next several weeks, the axons from the two eyes segregate into the eye-specific domains that are characteristic of the adult nucleus. Intraocular injection of tetrodotoxin prevents this process of segregation, showing that it depends on activity generated in the retina. This raises the question of what the source of the activity is and how it orchestrates segregation.

Because segregation occurs *in utero*, before the development of photoreceptors, it cannot be driven

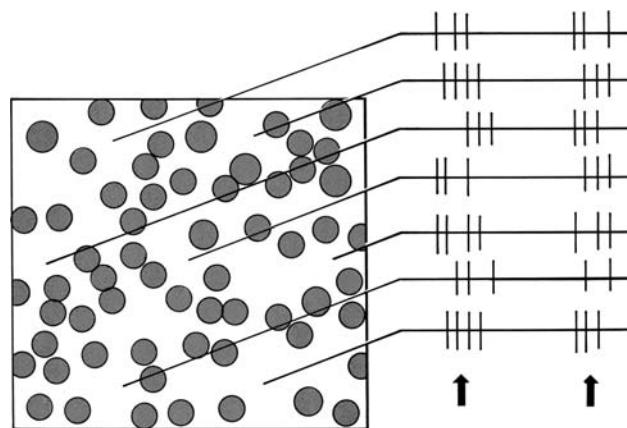


Fig. 11. Illustration of an experiment in which action-potential recordings were made from a whole-mount specimen of the fetal retina *in vitro*. Circles represent ganglion-cell outlines. Action potentials occur in spontaneous bursts (arrows) that are almost synchronous in widely separated regions of the retina. These local correlations in activity are believed to play a critical role in the sorting of retinal axons in the LGN. (Modified from Shatz CJ. The developing brain. Sci Am 1992;267:61.)

by photic stimulation. It appears that ganglion cells are spontaneously active during this period of fetal development. This activity is not random. Ganglion cells fire in quasi-synchronous waves that spread across the retina (Fig. 11). The origin of the wave and its direction of propagation may be random, but during each wave the activity in a ganglion cell is highly correlated with the activity in its nearest neighbors. Because these waves are generated independently in the two retinas, the activity patterns arising in the two eyes are not correlated with one another.

Segregation is believed to depend on a process of synaptic stabilization in which only retinal terminals that are active at the same time as their postsynaptic LGN target neuron are retained, a model first proposed by Donald Hebb in the 1940s; thus, the mnemonic “neurons that fire together wire together,” attributed to Sigrid Lowel and Wolf Singer. Connections that are modified according to this rule are said to employ Hebb synapses. According to the hypothesis, when a wave of retinal activity drives a postsynaptic LGN neuron, the active retinal inputs onto this neuron are consolidated. Because the activity from the two eyes does not occur in register, the inputs compete on a winner-takes-all basis until one input is retained and the other is eliminated, leading to complete segregation of the two inputs.

9.2. Segregation of Axons in Layer IV Is Influenced by Activity

Similar to the situation in the LGN, the afferents subserving the two eyes are initially intermingled in cortical layer IV and then segregate under the influence of activity (Fig. 12). To the extent that segregation occurs postnatally, the formation of ocular dominance columns can be affected by deprivation of normal-pattern vision. This is most dramatically demonstrated by monocular deprivation, in which the amount of light reaching the affected retina is only slightly decreased, but image formation is greatly impaired (e.g., experimentally by suturing the eyelid closed, or clinically by a unilateral congenital cataract). In experimental animals that have their eyes sutured shortly after birth and whose eyes remain sutured throughout the period of natural segregation, columns representing the open eye develop to be much wider than are columns representing the closed eye (Fig. 13). If deprivation is begun later, after the period of natural segregation, anatomic effects on

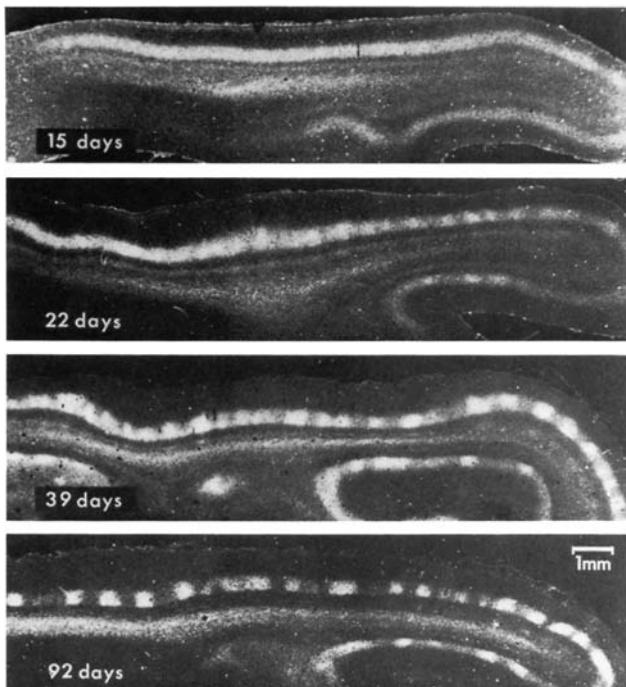


Fig. 12. Dark-field autoradiographs of the cat visual cortex cut in horizontal sections. In each case, one eye had been injected with ^3H -proline, and the animals were later sacrificed at the ages shown. At 15 days of age, the radioactivity is uniform in layer IV. Ocular-dominance columns segregate progressively over the next several weeks. (From LeVay S, Stryker MP. The development of ocular dominance in the cat. *Soc Neurosci Symp* 1979;483.)

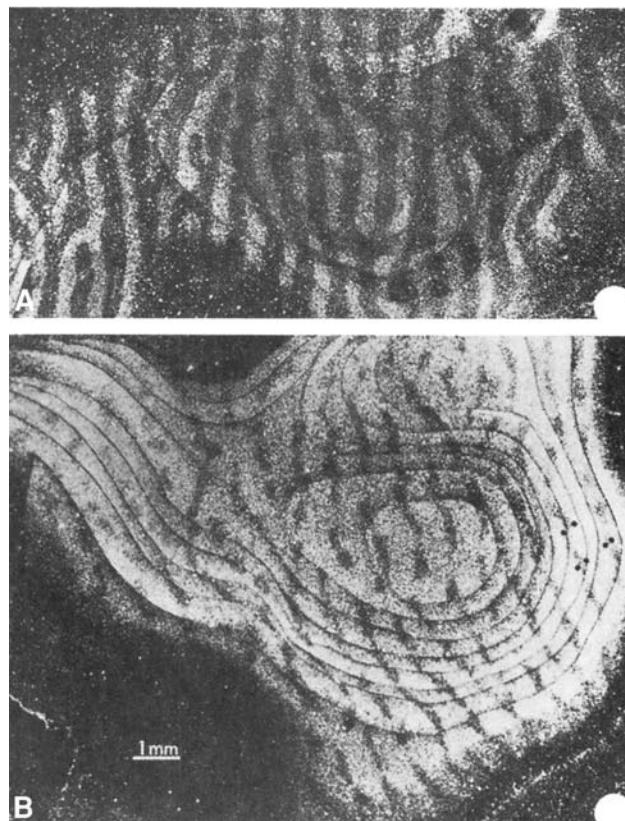


Fig. 13. Dark-field autoradiographs of tangential sections through layer IV of monkey striate cortex after injection of one eye with ^3H -proline. (A) Normal monkey. (B) Monkey that had been monocularly deprived for 18 months starting at 2 weeks of age. The nondeprived eye had been injected, revealing expanded ocular-dominance columns in layer IV. (From Wiesel TN. Postnatal development of the visual cortex and the influence of the environment. *Nature* 1982;299:583.)

LGN axon arbors are not observed in layer IV. Thus, a critical period exists for this type of plasticity.

Within this critical period, closing the previously open eye and opening the previously closed eye can reverse the anatomic effects of monocular deprivation. The shrunken ocular-dominance columns of the formerly closed eye expand, and the expanded columns of the formerly open eye shrink. This suggests that within the critical period, even after ocular-dominance column segregation appears to be anatomically complete, the afferents subserving the two eyes exist in a dynamic equilibrium that can still be disrupted by deprivation. At the end of the critical period, the afferents apparently lose their capacity for growth and retraction.

One correlate of the change in cortical ocular-dominance columns during the critical period is a change in the size of the neurons in the LGN that relay

information to the visual cortex. LGN cells deprived of normal visual input are visibly shrunken. This change in soma size is believed to reflect the decreased axonal arbors of these cells in layer IV. Curiously, shrinkage is observed only in the segment of the LGN where both retinas are represented. Cells in the monocular segment are largely unaffected by deprivation. In addition, destruction of part of the central retina in the nondeprived eye results in a region of the LGN that is relatively free of the effects of binocular competition, showing much less shrinkage of LGN neurons (Fig. 14). These observations suggest that the loss of territory in layer IV by afferents deprived of normal visual input is not caused by simple disuse but rather by a more active process of binocular competition that requires pattern vision in the open eye. Evidently, the activity in the open

eye actively promotes the synaptic disconnection of afferents that subserve the closed eye.

9.3. The Establishment and Maintenance of Binocular Connections Is Influenced by Visual Experience

The last connections to be specified during the development of the retinal-geniculate-striate pathway are those that subserve binocular vision. These are formed and modified under the influence of sensory experience during early postnatal life. Unlike the segregation of eye-specific domains, which evidently depends on asynchronous patterns of activity spontaneously generated by the two retinas, the establishment of binocular receptive fields depends instead on correlated patterns of activity that arise from the two eyes as a consequence of vision.

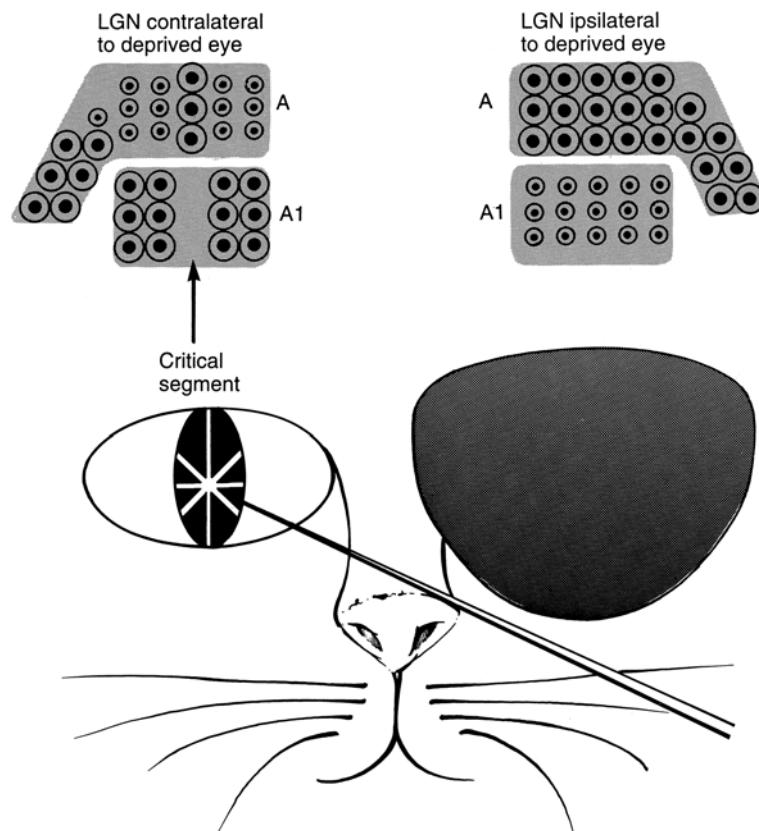


Fig. 14. Illustration of the critical segment in the experiment by R.W. Guillery, who found that cells in the monocular segment of the lateral geniculate layer A did not shrink like those in the binocular segment after monocular deprivation of the contralateral eye. To test the hypothesis that the shrinkage resulted from some competitive interaction of inputs arising from homotypic points in the two retinas, Guillery produced a lesion in the central region of the nondeprived retina. This removed the competition from the open eye in a critical segment of the lateral geniculate, and in this region, the cells in layer A did not shrink after monocular deprivation. Lateral geniculate cell size is believed to accurately reflect the extent of the axon arbor in cortical layer IV. These results supported the concept of binocular competition in the regulation of ocular-dominance columns in the visual cortex. (From Guillery RW. Binocular competition in the control of geniculate cell growth. Comp Neurol 1972;144:117.)

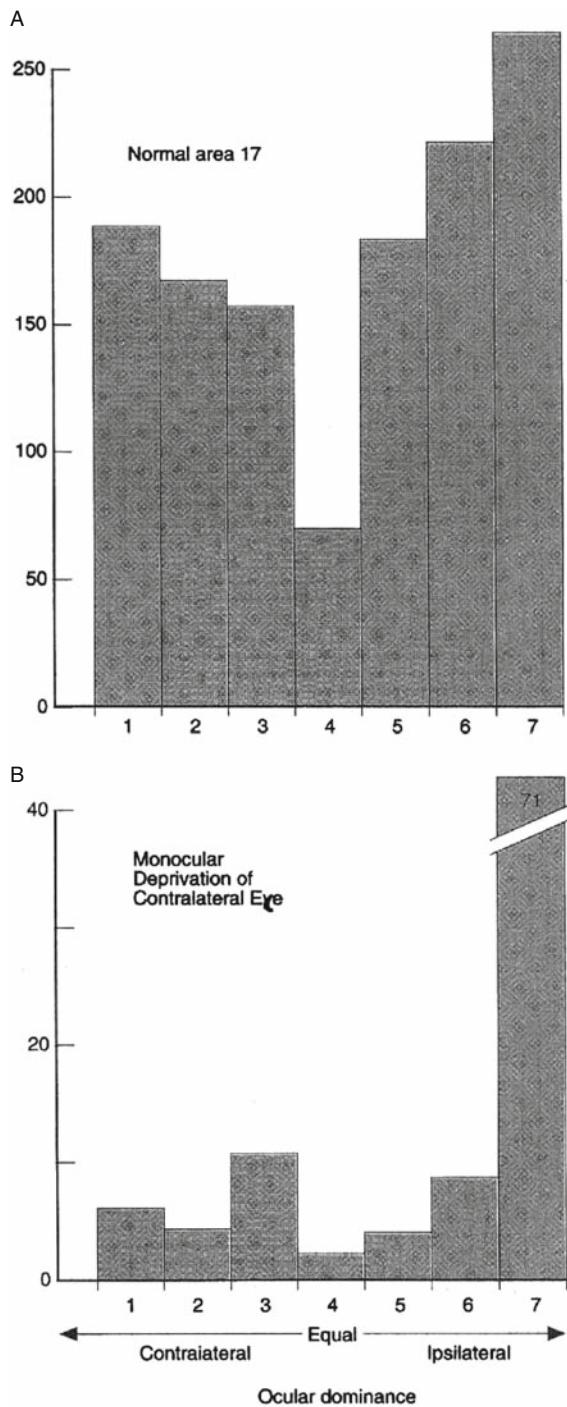


Fig. 15. The ocular-dominance shift after monocular deprivation. Illustrated are histograms of ocular-dominance data obtained from the striate cortex of (A) normal monkeys and (B) a monkey that had been monocularly deprived early in life. The bars show the number of neurons outside of layer IVc in each of the seven ocular-dominance categories. Cells in groups 1 and 7 are activated by stimulation of the left or right eye, respectively, but not both. Cells in group 4 are activated equally well by either eye. Cells in groups 2 and 3 and in groups 5 and 6 are binocularly activated, but they show a preference for the left or right eye, respectively. The histogram in (A) reveals that most neurons in the visual cortex of a normal animal are driven binocularly. The histogram in (B) shows that a period of monocular deprivation leaves few neurons responsive to the deprived eye. (Modified from Wiesel TN. Postnatal development of the visual cortex and the influence of the environment. *Nature* 1982;299:583.)

For example, bringing the patterns of activity from the two eyes out of register by monocular deprivation that replaces pattern vision in one eye with "white noise" profoundly disrupts the binocular connections in the striate cortex. Neurons outside of layer IV, which normally have binocular receptive fields, respond only to stimulation of the nondeprived eye after even a brief period of monocular deprivation. This change in the binocular organization of the cortex is known as an ocular dominance shift (Fig. 15).

These effects of monocular deprivation are not merely a passive reflection of the anatomic changes in layer IV. An ocular-dominance shift occurs in response to monocular deprivation initiated well beyond the period of susceptibility of LGN-axon arborization. However, this form of plasticity is also limited to a critical period of postnatal life. Although ocular-dominance plasticity in the cat peaks at 1 month of age and declines to very low levels by 3 to 4 months of age (Fig. 16), it is estimated that in human children this plasticity extends to about 10 years of age. These critical periods coincide with the times of greatest growth of the head and optical axes. Plasticity of binocular connections is probably required to maintain good binocular vision throughout this period of

rapid growth. The hazard associated with this activity-dependent fine-tuning is that these connections are also highly susceptible to deprivation.

Strabismus, or misalignment of the two eyes, also disrupts cortical binocularity. Visually evoked patterns of activity arrive at the cortex out of register, causing a total loss of binocular receptive fields although the two eyes retain equal representation in the cortex (Fig. 17). This is a clear demonstration that the disconnection of inputs from one eye occurs as the result of competition rather than disuse; the two eyes are equally active, but for each cell, a winner takes all. Strabismus, if produced early enough, can also sharpen the segregation of ocular-dominance columns in layer IV.

The changes in ocular dominance and binocularity after deprivation have clear behavioral consequences. An ocular-dominance shift after monocular deprivation leaves the individual (or test animal) visually impaired in the involved eye, and the loss of binocularity associated with strabismus completely eliminates stereoscopic depth perception. However, both of these effects can be reversed if they are corrected early enough in the critical period. The clinical lesson is that congenital cataracts or ocular misalignment must be corrected in early childhood, as soon as is surgically feasible, to avoid permanent visual disability.

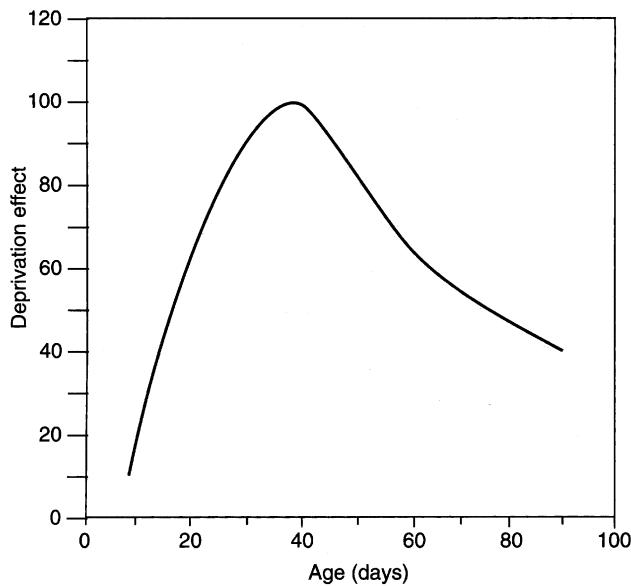


Fig. 16. Sensitivity of binocular connections in cat striate cortex to monocular deprivation at different postnatal ages. The deprivation effect is the percentage of neurons in area 17, whose responses are dominated by stimulation of the nondeprived eye. This critical period begins at about 3 weeks of age and declines to low levels after 3 months of age. (Modified from Dudek SM, Bear MF. A biochemical correlate of the critical period for synaptic modification in kitten visual cortex. *Science* 1989;246:673.)

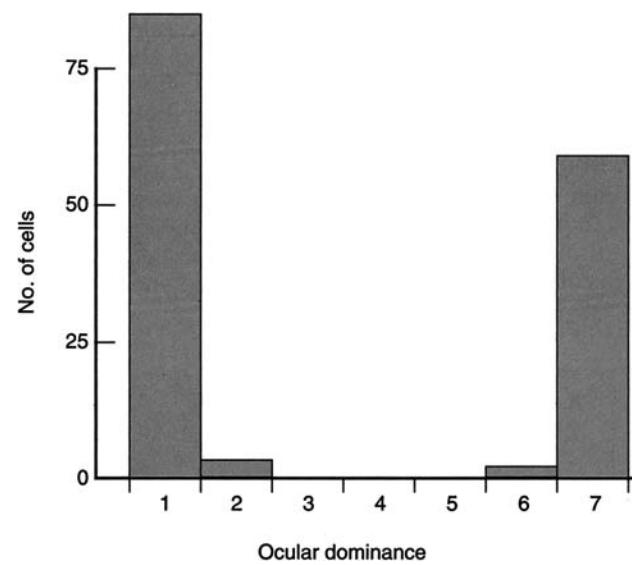


Fig. 17. Ocular-dominance histogram of cells recorded in the striate cortex of a 3-year-old strabismic monkey, in which the lateral rectus muscle of the right eye was sectioned at 3 weeks of age. Binocular cells are almost completely absent; the cells are driven exclusively by the right or the left eye. (Modified from Wiesel TN. Postnatal development of the visual cortex and the influence of the environment. *Nature* 1982;299:583.)

9.4. The Development and Modification of Binocularity Is Influenced by Extraretinal Factors

With increasing age, there appear to be additional constraints on the forms of activity that can modify cortical circuits. Before birth, spontaneously occurring bursts of retinal activity are sufficient to orchestrate aspects of address selection in the LGN and cortex. After birth, an interaction with the visual environment is of critical importance. However, even visually driven retinal activity may be insufficient for modifications of binocularity during this critical period. Such modifications seem to require that the patient (or test animal) attend to visual stimuli and use vision to guide behavior. For example, modifications of binocularity after monocular stimulation do not occur in anesthetized animals, although cortical neurons respond briskly to visual stimulation under this condition. These and related observations have led to the proposal that synaptic plasticity in the cortex requires the release of extraretinal “enabling factors” that are linked to the behavioral state. There is some evidence to suggest that this release may occur in response to eye movements.

Some data suggest that these enabling factors might be related to several modulatory systems converging at the level of the striate cortex, including the noradrenergic inputs from the locus coeruleus and the cholinergic inputs from the basal forebrain. These axonal projections have a trajectory that is distinct from the optic radiation from the LGN to the cortex. Surgical transection of these modulatory inputs in animal models substantially impairs ocular-dominance plasticity outside of cortical layer IV, although transmission in the retinal-geniculate-cortical pathway is apparently normal (Fig. 18). It is known that stimulation by acetylcholine and norepinephrine increase the excitability of cortical neurons, perhaps increasing the chance that they will generate action potentials in response to visual stimulation.

9.5. The Elementary Mechanisms of Synaptic Plasticity During the Critical Period May Involve NMDA Receptors

Much of the activity-dependent development of the visual system can be explained using Hebb synapses, particularly the development of binocular connections, in which afferents converging onto the same cell are consolidated only if they carry synchronous patterns of activity. The mechanisms by which consolidation of afferent connections is induced by synchronous firing derives from excitatory synaptic

transmission. The transmitter at all of the modifiable synapses (e.g., retinogeniculate, geniculocortical, and cortico-cortical) is likely to be an amino acid, glutamate, or aspartate, and is known to recruit a family of postsynaptic receptors called excitatory amino acid receptors. These receptors may be divided into two broad categories known as metabotropic and ionotropic. The metabotropic receptors are linked by G proteins to intracellular second-messenger systems. The ionotropic receptors are ion channels that allow passage of positively charged ions into the postsynaptic cell. These receptors may be further divided into two categories named for compounds that act as selective agonists at these sites: the AMPA (*a*-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor and the NMDA (*N*-methyl-D-aspartate) receptor. AMPA and NMDA receptors are colocalized at many synapses. Synaptic activation of the AMPA receptor activates a monovalent cation conductance that shows a linear current-voltage association with a reversal potential of approximately 0 mV. Activation of this receptor by glutamate stimulates an inward (i.e., depolarizing) current whose amplitude diminishes as the postsynaptic membrane is depolarized because of decreased driving force.

The NMDA receptor has two unusual features that differentiate it from the AMPA receptor. First, the NMDA-receptor conductance is voltage-dependent because of the action of Mg^{2+} at the channel. At the resting-membrane potential, the inward current through the NMDA receptor is interrupted by the movement of Mg^{2+} ions into the channel, where they become lodged. However, as the membrane is depolarized, the Mg^{2+} block is displaced from the channel, and current is free to pass into the cell. Substantial current through the NMDA-receptor channel requires concurrent release of glutamate by the presynaptic terminal and depolarization of the postsynaptic membrane. The other distinguishing feature of this receptor is that the NMDA-receptor channel conducts Ca^{2+} ions. The magnitude of the Ca^{2+} flux passing through the NMDA-receptor channel specifically signals the level of presynaptic and postsynaptic coactivation. It is believed that NMDA receptors in the cortex and LGN serve as Hebbian detectors of coincident presynaptic and postsynaptic activity and that Ca^{2+} entry through the NMDA-receptor channel triggers the biochemical mechanisms that modify synaptic effectiveness. Hebbian enhancement of synaptic effectiveness has been shown experimentally in the connections from layer IV onto layer III neurons in the visual cortex.

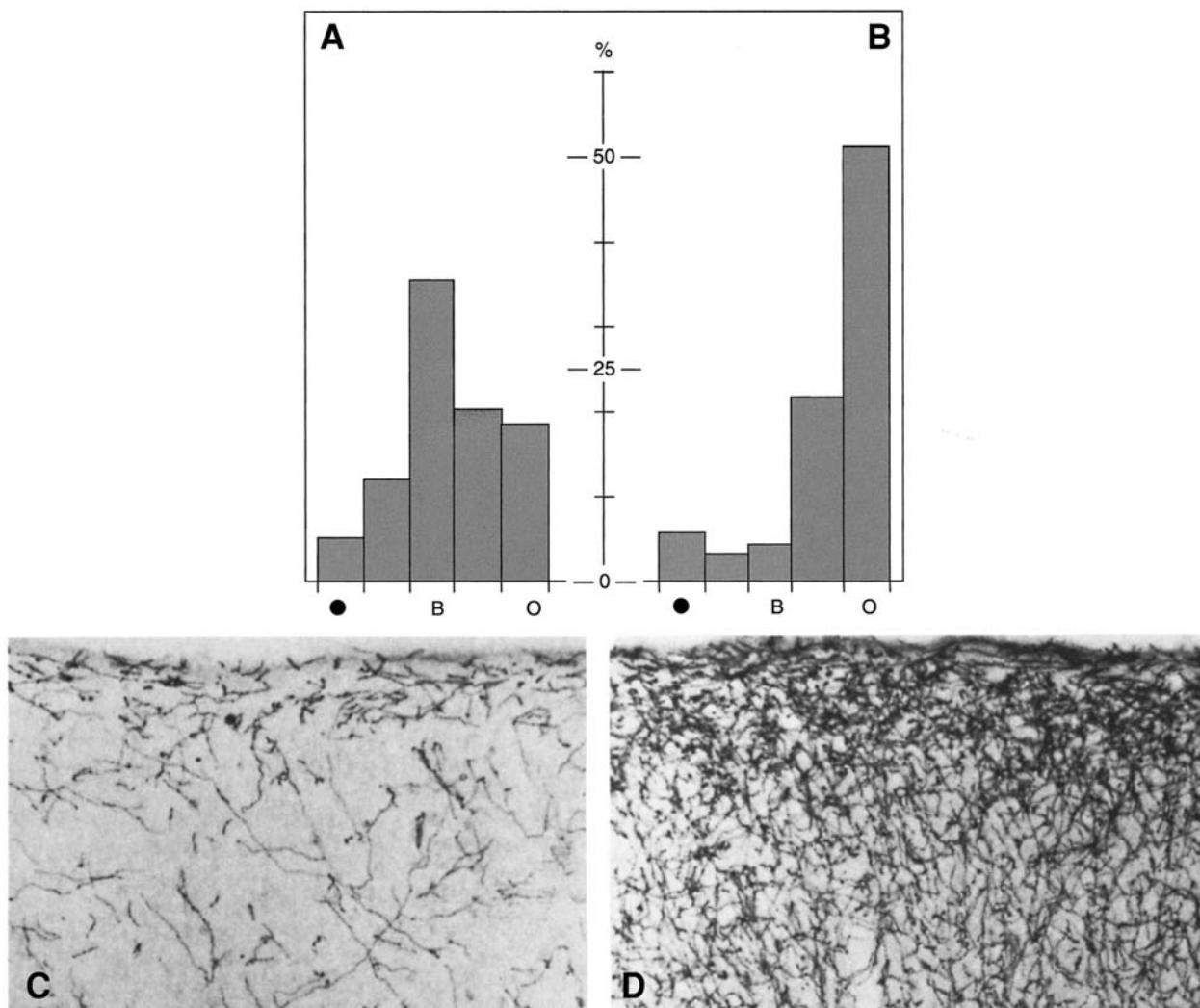


Fig. 18. Destruction of the modulatory noradrenergic and cholinergic inputs to the striate cortex interferes with the plasticity of binocular connections. **(A, B)** Percentages of cells in each of five ocular-dominance categories in striate cortex of monocularly deprived kittens. Open circles indicate the monocular open-eye group; filled circles indicate the monocular closed-eye group, and the x-axis B indicates the strictly binocular group. Data in **(A)** were obtained from the striate cortex ipsilateral to a unilateral transection of the cingulated bundle, a fiber tract that brings the modulatory inputs to the visual cortex. Data in **(B)** were obtained from the contralateral hemisphere where the modulatory inputs were intact. The ocular-dominance shift occurred only in the intact hemisphere. **(C, D)** Distribution of cholinergic axons in the striate cortex of a hemisphere with a lesion of the ascending modulatory fibers and of the contralateral hemisphere with these fibers intact, respectively. (From Bear MF, Singer W. Nature 1986;320:172.)

Pairing low-frequency stimulation of layer IV with intracellular depolarization of a cell in layer III results in a long-term potentiation (LTP) of the conditioned synapses (Fig. 19). This long-term potentiation is prevented by application of the drug 2-amino-5-phosphonovaleric acid (APV), an antagonist of the NMDA receptor. The theory that similar mechanisms contribute to naturally occurring synaptic remodeling is suggested by observations that application of APV *in vivo* can disrupt the natural segregation of

eye-specific inputs in the LGN, binocular competition in layer IV, ocular-dominance domains after monocular deprivation, and the modification of binocular connections in the superficial layers under a number of experimental manipulations of visual experience (Fig. 20).

The strong activation of NMDA receptors that occurs when presynaptic and postsynaptic neurons fire together is believed to account partly for why they wire together during visual-system development.

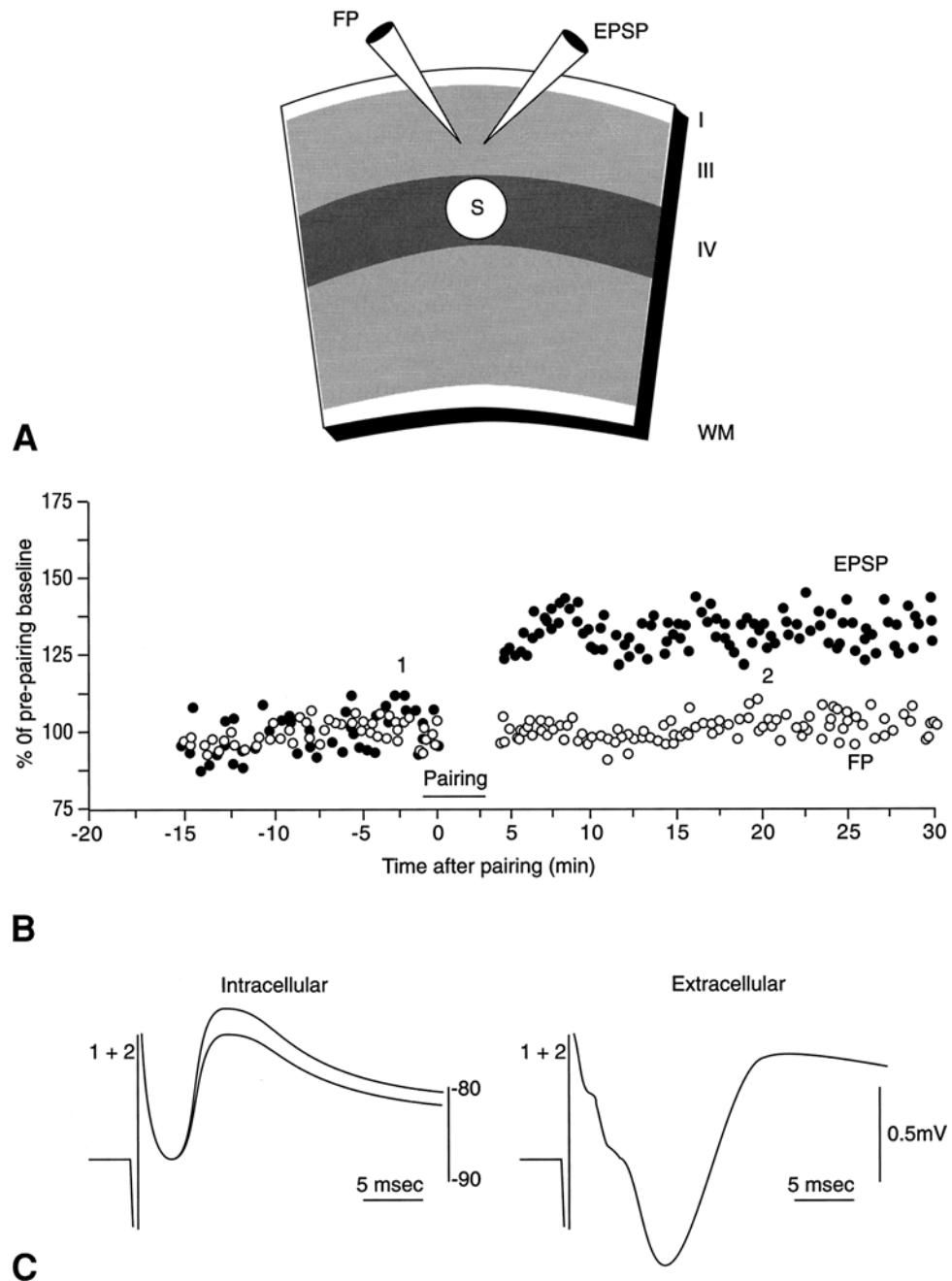


Fig. 19. Record of an experiment demonstrating Hebbian synaptic modification of the connections between layer IV and layer III in the rat visual cortex. **(A)** Layer IV was electrically stimulated (at the site marked *S*) every 30 s, and **(B, C)** the intracellular excitatory postsynaptic potential (EPSP) and extracellular synaptic responses in layer III were monitored. At the time indicated, the electrical stimuli to layer IV were paired with strong depolarization of the intracellularly recorded layer III neuron. As a consequence of this pairing, the intracellular response of the layer III neuron to the test stimulation of layer IV was potentiated. The extracellular response, which reflects the summed activity of a large population of synapses on many layer III neurons, is unchanged. FP, field potential.

However, the NMDA receptor does not function as a switch that is only “on” when input activity coincides with strong postsynaptic depolarization. Weak coincidences are signaled by lower levels of NMDA-receptor activation and less Ca^{2+} influx. Experiments

suggest that the lower level of Ca^{2+} admitted under these conditions triggers an opposite form of synaptic plasticity, long-term depression (LTD), by which the effectiveness of the active synapses is decreased. The maintenance of a connection formed during

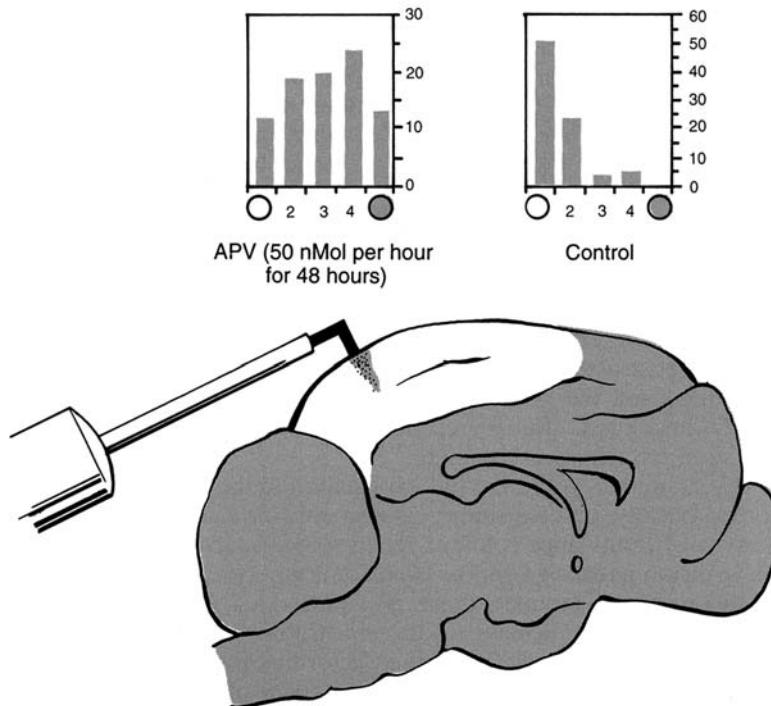


Fig. 20. Blockade of NMDA receptors in the visual cortex interferes with the ocular-dominance shift after monocular deprivation. Implanted small osmotic pumps delivered the NMDA-receptor antagonist 2-amino-5-phosphonovaleric acid (APV) directly to the visual cortex. At the same time as the infusion, the animal was monocularly deprived. No ocular-dominance shift was observed in the APV-treated cortex compared with controls. Conventions for these histograms are the same as used in Fig. 19. (Data from Bear MF, Kleinschmidt A, Gu Q, Singer W. Disruption of experience-dependent synaptic modifications in the striate cortex by infusion of an NMDA-receptor antagonist. *J Neurosci* 1990;10:909.)

development may depend on its success in evoking an NMDA receptor-mediated response beyond some threshold level. Failure to achieve this threshold leads to disconnection. Both processes depend on activity in the retinofugal pathway and on postsynaptic Ca^{2+} entry.

9.6. The Visual Cortex Is Plastic Beyond the Critical Period

In visual-system development, there are multiple critical periods. For example, the critical period for activity-dependent anatomic rearrangements of geniculate axonal arbors in layer IV ends much earlier than does the critical period for the experience-dependent modification of binocular connections. It is important to understand that the primary visual cortex of the adult brain is not immutable simply because it has aged beyond the critical period for a particular developmental process. Critical periods must be defined according to which type of intercellular interaction is being considered.

Modification of striate cortical circuits after a circumscribed lesion in the retina provides an example

of plasticity in the adult brain. The cortical neurons at the corresponding point in the retinotopic map initially fall silent and are unresponsive to any type of visual stimulation. However, in a period of a few weeks the same neurons again become visually responsive, but to the region of retina surrounding the lesion.

The retinal lesion causes a “filling in” of the cortical retinotopic map (Fig. 21). The anatomic substrate of this map plasticity is thought to be an adjustment in the effectiveness of horizontal projections that interconnect the layer III neurons in different parts of the retinotopic map. This demonstrates that cortical circuits can be modified by peripheral lesions well after the end of the critical period that is defined by the effects of monocular deprivation.

It is assumed that synaptic adjustments to more subtle changes in the sensory environment give the cortex a life-long capability to reorganize itself. The elementary mechanisms of synaptic plasticity, LTP and LTD, persist in the superficial layers of the adult striate cortex. It is likely that experience-dependent synaptic remodeling in the cortex is one mechanism

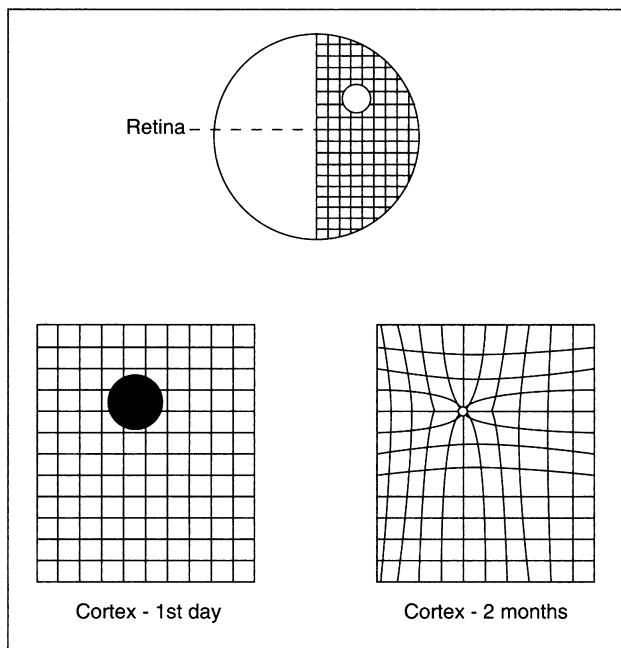


Fig. 21. Plasticity in the adult visual cortex. A lesion in the retina initially causes a patch of cortex to fall silent. However, these silent neurons later become responsive to stimulation of the surrounding areas of retina, causing the map to “fill in.” (Modified from Barinaga M. The brain remaps its own contours. *Science* 1992;258:216.)

that contributes to environmental adaptation and possibly to learning and memory in the adult brain.

9.7. Why Do Critical Periods End?

Although plasticity of visual connections persists in the adult brain, the dynamic range over which this plasticity occurs decreases with increasing age. Early in development, gross rearrangements of axonal arbors are possible, but in the adult, the plasticity appears to be restricted to local changes in synaptic efficacy. The adequate stimulus for evoking a change also appears to be increasingly constrained as the brain matures. An obvious example is the fact that patching one eye causes a profound alteration in the binocular connections of the superficial layers during infancy, but by adolescence, this type of experience fails to cause a lasting alteration in cortical circuitry.

There still is no satisfactory single explanation of why critical periods end. As more is learned about the elementary mechanisms of axonal pathfinding and synaptic plasticity, insights will be gained about how these processes are regulated. However, it is already possible to identify some of the rate-limiting factors that govern activity-dependent plasticity in the developing visual pathway.

One common feature in the establishment of connectivity in the LGN and cortex is the initial activity-independent establishment of widespread “exuberant” connections that are sculpted by activity to achieve their final form during a critical period. One explanation for the end of a critical period of, for example, ocular-dominance-column formation in layer IV is that, once segregation is complete and the afferents no longer contact the same postsynaptic cells, the substrate for the winner-takes-all competition is lost (Fig. 22). According to this view, monocular deprivation, by removing a competing input, only “saves” the initially widespread open-eye axonal arbors from being retracted. If it is assumed that axons lose the ability to grow after they have invaded their target structure and established their initial pattern of connectivity, the final state of connectivity is permanently “imprinted” after segregation (by loss of synapses and axon retraction) is complete.

This is unlikely to be the full explanation for the visual cortex, because reverse suture experiments have shown that ocular-dominance columns retain some capacity for reexpansion after segregation is anatomically complete. Another factor that limits the critical period in layer IV appears to be a loss of the capability for axonal elongation, which may be caused by changes in the ECM or surface glycoproteins expressed by the geniculate axons.

A third possible reason the critical period ends reflects changes in the elementary mechanisms of synaptic plasticity. There is evidence that some EAA receptors change during postnatal development. For example, it has been shown that EAA-stimulated phosphoinositide turnover that is mediated by one of the metabotropic receptors peaks in the visual cortex when binocular connections are most susceptible to

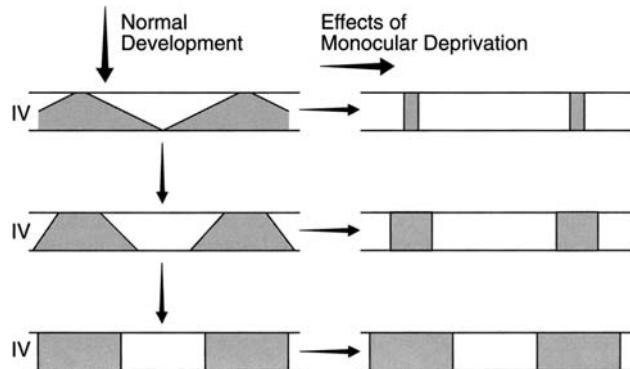


Fig. 22. Segregation of ocular-dominance columns in layer IV at three times during postnatal development and the effects of monocular deprivation initiated at these same times.

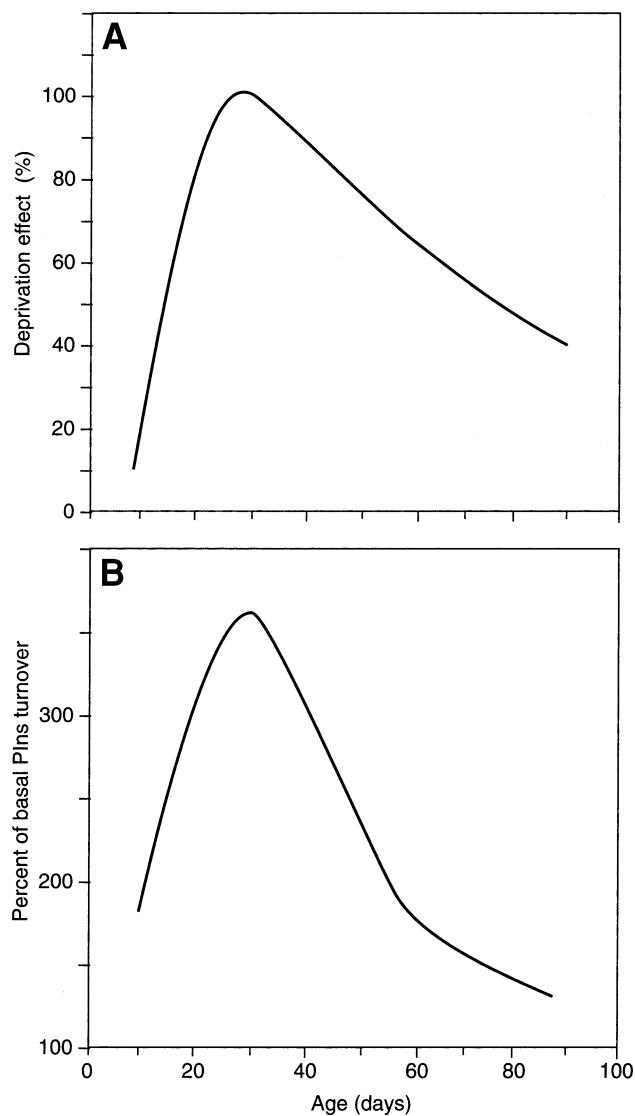


Fig. 23. (A) Sensitivity of binocular connections in striate cortex to eyelid suture at different postnatal ages. (B) Phosphoinositide turnover stimulated by an excitatory amino acid in synaptoneuroosomes prepared from kitten striate cortex at different postnatal ages. These data suggest that excitatory synaptic transmission during this critical period is characterized by unique patterns of second messenger activity. (Modified from Dudek SM, Bear MF. A biochemical correlate of the critical period for synaptic modification in the kitten visual cortex. *Science* 1989;246:673.)

monocular deprivation and then virtually disappears at the end of this critical period (Fig. 23). The effectiveness of NMDA receptors appears to be down-regulated in layer IV at the same time as the end of the critical period for ocular-dominance column segregation. This change in effectiveness can be detected at the single-channel level and may result from a developmental change in the NMDA-receptor subunit composition.

As development proceeds, certain types of activity may be filtered by successive synaptic relays until they no longer activate NMDA receptors or other elementary mechanisms sufficiently to trigger plasticity. The neuromodulators acetylcholine and norepinephrine facilitate synaptic plasticity in the superficial cortical layers and may do this by enhancing polysynaptic intracortical transmission. A decline in the effectiveness of these neuromodulators or a change in the conditions under which they are released may contribute to the decline in plasticity. There is some evidence that supplementing the adult cortex with norepinephrine can restore some degree of modifiability. There is also evidence that intrinsic inhibitory circuitry is late to mature in the visual cortex. Consequently, patterns of activity that may have gained access to modifiable synapses in superficial layers early in postnatal development may be damped by inhibition in the adult.

Whatever the reason for the decline in synaptic plasticity at the end of the critical periods, it is clear that the duration of the critical periods is not always locked to a certain postnatal age. For example, rearing kittens in complete darkness appears to slow the critical period for modification of binocular connections (Fig. 24). Dark-rearing also slows segregation of ocular dominance columns and some of the changes

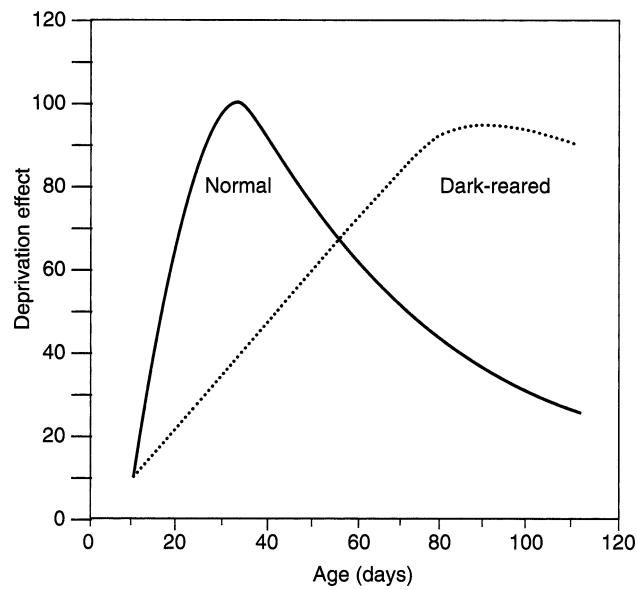


Fig. 24. Raising cats in complete darkness before visual experience with one eye open slows the critical period for the modification of binocular connections. (Data from Mower GC. The effect of dark rearing on the time course of the critical period in cat visual cortex. *Dev Brain Res* 1991;58:151.)

in EAA-receptor properties. It seems that the duration of this critical period may be measured by the history of stimulation rather than by age.

10. DEFINITION OF THE OCULOMOTOR SYSTEM

The oculomotor system has the dual tasks of maintaining stability of eye position and directing eye movements toward novel features of our environment. This system operates under very fine tolerances. The fovea, the region of highest visual acuity on the retina, “sees” only an area about the size of a quarter held at arm’s length. Thus, the eyes must be precisely directed toward an object of interest and held in that position for precise and clear vision. Drift of the eyes produces blurred vision, and misalignment of both eyes (strabismus) causes double vision, or diplopia.

The oculomotor system is known as the efferent limb for the visual system and vestibular system, but it is important to remember that eye movements are also directed by the senses of hearing or touch. The five distinct eye movement systems described here are responsible for maintaining stability of eye position and directing the eyes to new targets. The extraocular muscles mediate all movements of the eyes and are characteristic to all of the eye-movement systems. These muscles are controlled by precise connections with several brain-stem structures that are related to eye movements in the vertical and horizontal planes. Extraocular muscle-fiber types and their elegant organization in the extraocular muscles are unmatched by any of the skeletal muscles that are controlled by spinal cord motor systems. Because the premotor structures that control eye movements also have connections with motor neurons in the cervical spinal cord that innervate muscles of the neck, the motor behavior produced by these structures is known as gaze. Gaze is a combination of coordinated eye and head movements and is the result of a complex interaction between the visual and vestibular systems that allows us to see clearly and precisely.

The mechanisms related to eye movements and gaze can best be appreciated by understanding the brain stem, cerebellar, and cortical connections of the pre-oculomotor structures. It is sometimes clinically possible to separate the various visual and vestibular components so that lesions in different portions of the cerebral cortex, the brain stem, and the cerebellum produce specific eye movements, or gaze deficits. This chapter will link the neuroanatomic pathways to the

five types of eye-movements, and illustrate the consequences of lesions at various points in eye-movement control pathways.

11. TYPES OF EYE MOVEMENTS

Eye movements are classified into five categories: vestibular, optokinetic, smooth pursuit, saccadic, and vergence. Of these, the vestibular, optokinetic, smooth pursuit, and saccadic eye movements are *conjugate movements*, in which both eyes move in the same direction at the same time. Conjugate eye-movement types share some components of the brain-stem neural circuitry. Vergence eye movements are *disjunctive movements*, in which the eyes move in opposite directions at the same time. This is achieved by using very different neural control mechanisms than those used for conjugate eye movements. Vergence and conjugate eye-movement systems overlap only in their sharing of the same motor neurons and extraocular muscles.

Movements of the head that activate the semicircular canals elicit *vestibular eye movements*. The *vestibulo-ocular reflex* (VOR) is a compensatory eye movement that replicates a head movement, but in the opposite direction. The function of the VOR is to maintain the stability of the visual field while the head moves. For example, rotation of the head to the right produces a compensatory conjugate eye movement to the left so that images in the visual field remain stationary on the retina. To understand the interaction of the VOR and vision, hold your thumb out in front and turn both head and arm while maintaining focus on your thumb. Note that your thumb stays in focus while the rest of the world is blurred as you *cancel* the VOR. If you did not have a VOR, any movement of the head would cause blurring of vision in the same way that moving a camera blurs the image on the film. The VOR works best for brief or rapid movements of the head, as the semicircular canals are best at detecting these types of movements.

Optokinetic eye movements are tracking movements elicited by movement of the entire visual field, such as occurs while looking out of the window of a moving train. The *optokinetic reflex* is the smooth eye movement that tracks a moving stimulus. *Optokinetic nystagmus* (OKN) is characterized by a slow-phase eye movement in the direction of a moving stimulus and a quick-phase return eye movement in the opposite direction when the excursion limit of the oculomotor range has been reached. The optokinetic system works synergistically with the vestibular

system to stabilize images in the visual field on the retina during movements of the head. The optokinetic reflex effectively compensates for those types of head movements that are not detected well by the vestibular apparatus—sustained or slow movements of the head.

Smooth-pursuit eye movements track images that move across the visual field. These movements maintain the focus of moving targets in the visual field on the fovea of the retina. Smooth-pursuit eye movements that are accompanied by movement of the head in the same direction require suppression or cancellation of the VOR.

Saccadic eye movements are typically rapid scanning movements that change foveal fixation from one point in the central visual field to another point in the periphery. Saccades are used as you skip from word to word in reading this book or in scanning a picture but also replace or augment smooth pursuit when an image moves too rapidly across the visual field. Intentional saccades, such as those made to a remembered target, are differentiated from reflexive saccades, which are made in response to a novel object that appears in the peripheral visual field.

Vergence eye movements are associated with changing the point of foveal fixation from a distant object to a near object. Vergence movements are disjunctive, because they are produced by contraction of the medial rectus muscles in both eyes. Vergence eye movements also are associated with changes in the shape of the lens of the eye (e.g., accommodation) and constriction of the pupil (e.g., miosis) as a part of the process is known as the near triad or near response.

12. EXTRAOCULAR MUSCLES

Regardless of the category of eye movement, all changes in eye position result from coordinated contraction and relaxation of extraocular muscles. There are six extraocular muscles responsible for eye movements: the superior rectus, lateral rectus, medial rectus, inferior rectus, superior oblique, and inferior oblique muscles. The superior and inferior recti are the primary elevators and depressors of the eye, respectively. The lateral rectus abducts and the medial rectus adducts the eye. Actions of the oblique muscles are more complex, but the superior oblique is the primary intorter (inward rotation of the eye) and the inferior rectus is the primary extorter (outward rotation). Damage to individual extraocular muscles, or to one of the three cranial nerves involved in eye movement, is easily detected by routine

ophthalmic examination. Upward deviations of the eyes are known as hypertropias, downward deviations are known as hypotropias, and lateral deviations are known as exotropias. Medial deviations are known as esotropias. An additional extraocular muscle, the levator palpebrae superioris, elevates the upper eyelid. Damage to the levator muscle or its nerve results in drooping of the eyelid, or ptosis.

13. EXTRAOCULAR MOTOR NUCLEI

Motor neurons in the extraocular motor nuclei (e.g., cranial nerves III, IV, and VI) are the final common pathway on which inputs converge from several brain-stem premotor structures that are related to the control of different types of eye movements. The cranial nerves with which these motor neurons are associated provide *general somatic efferent* innervation of the extraocular muscles.

13.1. The Oculomotor Complex Contains Somatic and Visceral Motor Neurons

The somatic division of the oculomotor complex (e.g., cranial nerve III) contains motor neurons that innervate the ipsilateral medial rectus, inferior rectus, and inferior oblique muscles and innervate the superior rectus and levator palpebrae superioris muscles bilaterally with contralateral predominance. Axons of the superior rectus and levator palpebrae superioris decussate in the vicinity of the oculomotor nucleus before coursing ventral to exit the brain stem with the remainder of the third cranial nerve. The oculomotor nerve then exits the brain stem from the ventral surface of the mesencephalon, through the interpeduncular fossa. At this location, the nerve passes between the superior cerebellar and posterior cerebral arteries, a clinically important anatomic configuration. Note the difference between oculomotor nucleus lesions, which would affect eye movements bilaterally, and oculomotor nerve lesions, which affect only the ipsilateral eye.

The motor neurons that innervate different muscles are arranged in a precise topographic organization within the oculomotor nucleus. The medial rectus and inferior rectus motor neurons are in close proximity to each other, and the superior rectus and levator palpebrae superioris motor neurons occupy adjacent subdivisions. Inferior oblique motor neurons are located in the vicinity of superior rectus motor neurons. This arrangement is largely a reflection of the synergistic actions of the muscles and the common inputs of the different populations of motor

neurons. Levator palpebrae superioris motor neurons are considered to occupy the *caudal central nucleus* subdivision of the oculomotor complex.

In addition to motor neurons, the somatic oculomotor nucleus and the overlying supraoculomotor region contain *internuclear neurons*, which have descending brain-stem connections with the abducens nucleus and facial nucleus. At least some of the internuclear neurons are contacted by motor neuron axon collaterals and may be involved in the coordinated activity of extraocular and facial muscles, which can occur during blinking.

The *anteromedian, Edinger-Westphal nuclei* are a collection of midline *preganglionic parasympathetic neurons* that overlie the rostral portion of the somatic oculomotor nucleus and curve ventrally rostral to the somatic nucleus. An additional population of autonomic neurons lies along the midline, between the somatic portions of the oculomotor nucleus. The *general visceral efferent* axons of these neurons course via the third cranial nerve to synapse in the ciliary ganglion, with postganglionic fibers distributed primarily to the sphincter pupillae muscle of the iris and ciliary body. The postganglionic fibers that innervate the sphincter pupillary muscles are related to the *pupillary light reflex*, and those that innervate the ciliary body control lens accommodation. Both autonomic and somatic divisions of the oculomotor nucleus function together in the viewing of near objects—the *near triad*, consisting of convergence, accommodation, and pupillary constriction. Another population of coexistent neurons located in the same region projects to the upper thoracic spinal cord (the site of preganglionic sympathetic neurons for the head and neck) and may be involved in the coordination of parasympathetic-constriction and sympathetic-dilation control of pupillary function.

The combination of somatic and parasympathetic components in the third cranial nerve forms the basis for characteristic deficits that are associated with a third-nerve palsy. The loss of parasympathetic innervation is manifested by pupillary dilatation (mydriasis), with a complete loss of the direct and consensual light reflexes for the affected eye, and anisocoria, or inequality of pupil sizes. Damage to the somatic portion of the oculomotor nerve produces an abnormal deviation of the eye, or strabismus. In the resting position, the apparent motility deficits are manifested as exotropia (external strabismus caused by an unopposed lateral rectus) and ptosis (drooping eyelid). The loss of innervation to the vertical eye muscles becomes apparent only when the

patient is asked to move the eyes upward or downward. Routine ophthalmic examinations test movements of the eyes in each direction, starting from different positions of gaze, and can precisely diagnose the site of brain stem or nerve lesions.

13.2. The Trochlear Nucleus Innervates the Contralateral Superior Oblique Muscle

The trochlear nucleus (cranial nerve IV) contains motor neurons that predominately innervate the contralateral superior oblique muscle, but approximately 10% of the motor neurons innervate the ipsilateral muscle. The axons exit the dorsomedial portion of the nucleus, course around the medial longitudinal fasciculus and periaqueductal gray, decussate in the anterior medullary velum, and exit the dorsal surface of the brain stem immediately caudal to the inferior colliculus. The trochlear nerve is the only one of the cranial nerves to exit from the dorsal surface of the brain stem. The small size of the fourth cranial nerve and its anatomic relationship to the tentorium cerebelli make it especially vulnerable clinically in trauma. Lesions of the fourth nerve (e.g., fourth-nerve palsy) are associated with an overacting inferior oblique muscle, so that the affected eye is higher (hypertropic) and extorted. If the lesion is bilateral, then a V-shaped pattern is seen—the eyes move together when looking down and apart when looking up.

13.3. The Abducens Nucleus Is the Center for Conjugate Horizontal Eye Movement

The abducens nucleus is composed of both motor neurons and interneurons that collectively participate in conjugate horizontal movements of the eyes (Fig. 25). Approximately 70% of the neurons of the abducens nucleus (e.g., cranial nerve VI) are abducens motor neurons that innervate the ipsilateral lateral rectus muscle. Their axons exit the ventral surface of the brain stem at the pontomedullary junction, just lateral to the pyramids as they emerge from the basilar pons. The sixth cranial nerve has the longest intracranial course of any of the cranial nerves. Because of its size it is clinically vulnerable (e.g., sixth-nerve palsy, which produces an exotropia), especially in the regions where it courses ventral to the basilar pons and where it traverses the petrous portion of the temporal bone.

The remaining 30% of neurons in the abducens nucleus are abducens internuclear neurons, whose axons cross the midline at the level of the abducens nucleus, ascend in the contralateral medial longitudinal

Control of Conjugate Horizontal Gaze

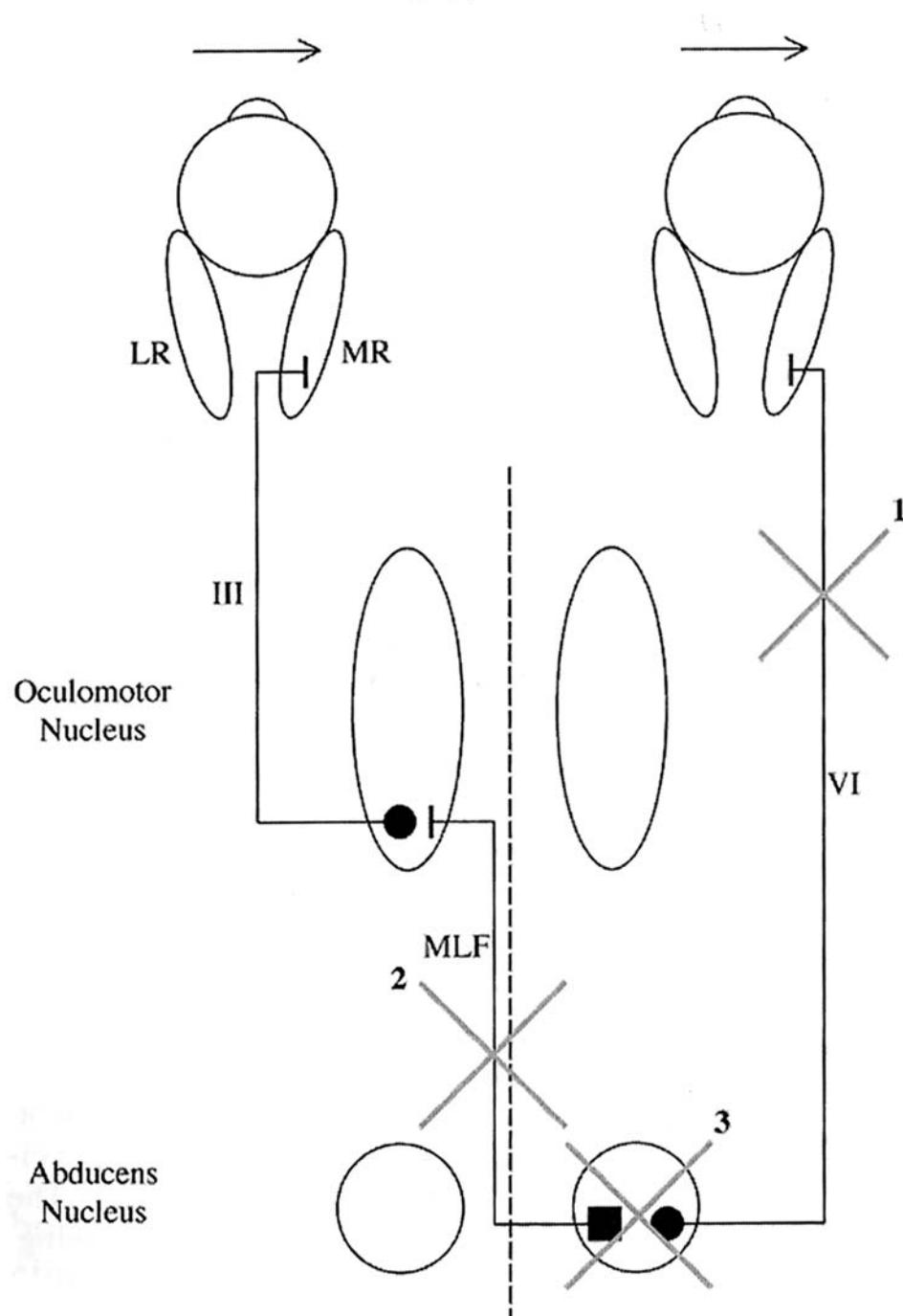


Fig. 25. The organization of conjugate horizontal eye movements. Medial and lateral rectus motor neurons (*filled circles*) are shown projecting through cranial nerves III and VI, respectively, to innervate the medial (MR) and lateral rectus (LR) muscles. Abducens internuclear neurons (*filled square*) send their axons across the midline and up the medial longitudinal fasciculus (MLF) to target contralateral medial rectus motor neurons. In this way, conjugate horizontal signals are sent equally to abducens motor neurons and abducens internuclear neurons, which then relay the signals faithfully to contralateral medial rectus motor neurons. Thus, activation of the ipsilateral abducens nucleus causes abduction of the ipsilateral eye and adduction of the contralateral eye. Because of the organization of horizontal gaze, there are differing consequences of abducens nerve (1), MLF (2), and abducens nucleus (3) lesions (see Section 13.3).

fasciculus (MLF), and establish extensive excitatory synaptic connections with medial rectus motor neurons in the contralateral oculomotor nucleus. The abducens nucleus is known as the *center for conjugate horizontal eye movement*, because it controls the ipsilateral lateral rectus muscle directly and the contralateral medial rectus muscle indirectly. This arrangement allows the abducens nucleus to elicit co-contraction of the lateral rectus muscle on the ipsilateral side and the medial rectus on the contralateral side, resulting in movement of both eyes in the ipsilateral direction.

Lesions of the abducens nucleus and the abducens nerve produce markedly different deficits in ocular motility (Fig. 25). Because the abducens nucleus contains both motor neurons and internuclear neurons, a lesion involving the nucleus produces paralysis of conjugate horizontal eye movements toward the side of lesion (lesion 3 in Fig. 25). Lesions of the sixth nerve are manifested by esotropia (e.g., internal strabismus caused by unopposed medial rectus) at rest and a paralysis of ipsilateral attempted abduction (lesion 1 in Fig. 25). Lesions of the MLF cause the syndrome of *internuclear ophthalmoplegia*, resulting in loss of adduction (medial rectus activation) during conjugate movements of the eyes (lesion 2 in Fig. 25). Internuclear ophthalmoplegia is not a rare occurrence, frequently resulting from a stroke or multiple sclerosis. A more complete explanation of internuclear ophthalmoplegia appears in Sections 14.1, 19.1.2, and the clinical correlation.

14. PRE-OCULOMOTOR NUCLEI

Four brain-stem premotor areas are individually significant for the control of eye movement and gaze in the vertical and horizontal planes. These structures have direct, monosynaptic connections with motor neurons in the extraocular motor nuclei.

14.1. The Vestibular Nuclei Control the Vestibulo-ocular Reflex

The VOR originates from the semicircular canals in the ear (see Chapter 24). In its simplest form, this reflex is mediated by an arc of three neurons—primary vestibular neuron to secondary vestibular neuron to eye-movement motor neuron. The superior vestibular nucleus and rostral portions of the medial and inferior vestibular nuclei receive afferents from semicircular canal-related primary vestibular axons and centrally from the flocculus and fastigial nuclei of the cerebellum. Canal-specific efferent projections of

the second-order vestibular neurons target motor neurons in the extraocular motor nuclei, thereby providing the basis for the VOR. Each semicircular canal is related to two pairs of muscles in each eye through reciprocal excitatory and inhibitory synaptic connections with the extraocular motor neurons. The basis of this interaction is explained by the relationship of the spatial orientation of the semicircular canals with the pulling actions of the extraocular muscles.

Axons from second-order anterior and posterior canals-related superior vestibular neurons ascend the ipsilateral MLF and are inhibitory to all vertical motor neurons in the oculomotor and trochlear nuclei (Fig. 26). Axons from second-order anterior canal-related neurons in the superior vestibular nucleus and from posterior canal-related neurons in the medial and inferior vestibular nuclei ascend the contralateral MLF and are excitatory to all vertical motor neurons in the oculomotor and trochlear nuclei. Horizontal canal-related medial or inferior

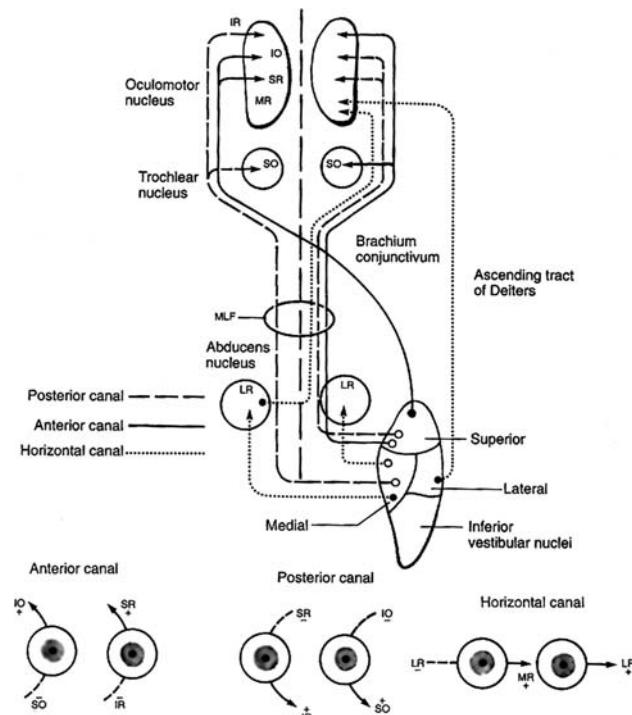


Fig. 26. The vestibulo-ocular pathways that relate each of the semicircular canals to the activation of specific pairs of extraocular muscles. Excitatory neurons are indicated by filled circles; inhibitory neurons are indicated by open circles. Excitatory fibers ascend contralateral to their cells of origin, and inhibitory fibers ascend ipsilateral to their cells of origin. The actions of extraocular muscles influenced by activation of the individual canals are indicated at the bottom of the drawing.

vestibular neurons are excitatory to contralateral abducens neurons (both motor neurons and inter-nuclear neurons) and inhibitory to ipsilateral abducens neurons. Medial rectus motor neurons in the oculomotor nucleus receive the majority of their vestibular inputs via the MLF. However, the medial rectus motor neurons also receive a small direct ipsilateral excitatory input from neurons located in the ventral portion of the lateral vestibular nucleus, whose axons ascend via the ascending tract of Deiters, which is lateral to the MLF.

The basic three-neuron reflex arc (e.g., first-order vestibular ganglion neuron→second-order vestibular nucleus neuron→extraocular motor neuron) is necessary but insufficient for the normal functional operation of the VOR. When a compensatory eye movement is made in response to rotation of the head, the head velocity signals of vestibular neurons are incapable of maintaining gaze in the new position. The prepositus hypoglossi nucleus, which is

located in the periventricular dorsal aspect of the medulla extending from the rostral pole of the hypoglossal nucleus to the abducens nucleus, plays a fundamentally important role in gaze holding (Fig. 27). The prepositus hypoglossi nucleus has extensive reciprocal connections with the vestibular nuclei and is regarded as the *neural integrator* responsible for converting the head velocity signals of vestibular neurons to eye-position signals that are carried by extraocular motor neurons. Damage to the prepositus hypoglossi nucleus interferes with integrator function, making it difficult to maintain gaze positions. Consistent with its function related primarily to horizontal eye movements, the excitatory and inhibitory connections of prepositus hypoglossi neurons are directed predominately to the abducens nucleus. A similar neural integrator function has been postulated for the neurons in the interstitial nucleus of Cajal, in the rostral midbrain, which controls vertical gaze.

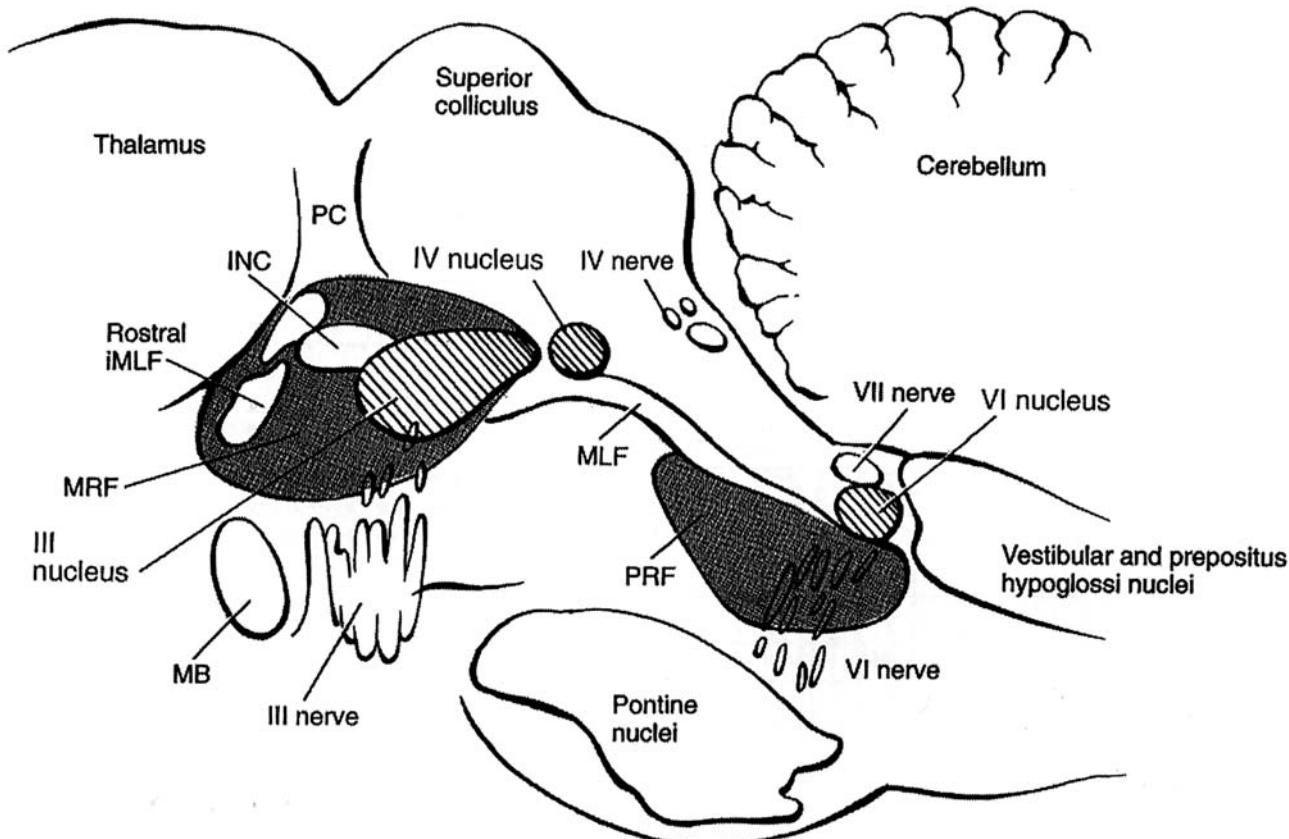


Fig. 27. A midsagittal view of the brain stem, indicating the locations of premotor neurons that are related to the control of gaze. The mesencephalic reticular formation (MRF) rostral to the oculomotor (III) nucleus contains the interstitial nucleus of Cajal (INC) and the rostral interstitial nucleus of the medial longitudinal fasciculus (rostral iMLF); both of these are related to the control of vertical upward and downward gaze. The paramedian pontine reticular formation (PRF) rostral and ventral to the abducens (VI) nucleus contains neurons that are related to the control of horizontal gaze. MB, mamillary body.

Lesions of the vestibular nerve or nuclei and of the MLF produce quite different deficits. Vestibular nerve or nucleus lesions are characterized by spontaneous nystagmus, an enhanced vertical nystagmus induced by caloric stimulation of the contralateral ear, and positional nystagmus. These deficits underscore the importance of balanced inputs from the semicircular canals and otolith organs on both sides. However, deficits associated with peripheral lesions are only transient because of compensation mediated by commissural connections between the vestibular nuclei on each side.

Lesions of the MLF in the pons and caudal midbrain, as noted previously, are responsible for the clinical syndrome of *internuclear ophthalmoplegia* (Fig. 25). This syndrome is characterized by paralysis of ipsilateral adduction on attempted conjugate horizontal eye movements to the opposite side and nystagmus in the abducted eye, but the preservation of vergence eye movements. In this case, the motor nerve innervation of the medial rectus and lateral rectus muscles is intact and muscle function is normal, but the axons of abducens internuclear neurons have been disrupted, and signals related to conjugate horizontal eye movements are not relayed to the oculomotor nucleus. Vergence eye movements are unaffected, because the premotor neurons and motor neurons that are responsible for these movements are located in the midbrain, rostral to the lesion.

14.2. The Mesencephalic Reticular Formation Controls Vertical Gaze

The region of the mesencephalic reticular formation in the vicinity of the oculomotor complex contains two structures that are intimately related to the control of vertical upward and downward gaze (Fig. 27). The *rostral interstitial nucleus of the medial longitudinal fasciculus* is located at the junction of the mesencephalon and diencephalon, lateral to the periventricular gray in the region of the subthalamus and the field H of Forel. Extending caudally from this location into the rostral midbrain, the *interstitial nucleus of Cajal* is lateral to the MLF at the level of the rostral portion of the somatic and visceral oculomotor nuclei. Neurons in both structures have afferent synaptic connections with the superior colliculus and the vestibular nuclei. Excitatory and inhibitory efferent connections are established bilaterally with vertical motor neurons in the oculomotor nucleus and trochlear nuclei (Fig. 28). Contralateral projections that are related

specifically to vertical upward eye movements cross the midline through the *posterior commissure*. The rostral interstitial nucleus of the MLF contains burst neurons that discharge before the initiation of vertical saccadic eye movements. This region is physiologically differentiated from the tonic neurons in the interstitial nucleus of Cajal, whose activity is related to eye position. Many of the neurons in these structures also project to the spinal cord, forming the basis for their role in the control of vertical gaze.

Lesions of the rostral interstitial MLF at the mesodiencephalic junction produce paralysis of vertical upward or downward gaze (e.g., Parinaud's syndrome), and direction depends on the extent of the lesion. Lesions of the posterior commissure have a selective effect on vertical upward saccadic eye movements and produce deficits in the pupillary light reflex as a result of the proximity to the pretectal area (e.g., pretectal syndrome).

14.3. The Pontine Reticular Formation Is the Center for Conjugate Horizontal Gaze

The paramedian zone of the pontine reticular formation in the vicinity of the abducens nucleus contains neurons that discharge with a burst of activity before the initiation of conjugate horizontal gaze. The *nucleus reticularis pontis caudalis* is rostral to the abducens nucleus and contains *excitatory burst neurons* that project to ipsilateral abducens neurons and to the cervical spinal cord. *Inhibitory burst neurons* are located caudal to the abducens nucleus and project to contralateral abducens neurons. Both populations of neurons receive inputs from the superior colliculus and vestibular nuclei (Fig. 28). A third type of neuron, known as the *omnipause neuron*, is also located in the vicinity of the burst neurons. The tonic activity of omnipause neurons inhibits the burst neurons, except during saccades. Omnipause neurons then serve as a gating mechanism that prevents spurious saccades. The importance of this function is seen in lesions of the omnipause neurons, which produce constant saccadic oscillations of the eyes (oscillopsia). Collectively, the region of the paramedian pontine reticular formation is known as the *center for conjugate horizontal gaze*. Lesions of this area result in paralysis of ipsilateral conjugate horizontal gaze (eye plus head movements), in contrast with lesions of the abducens nucleus, which affect only conjugate horizontal eye movements.

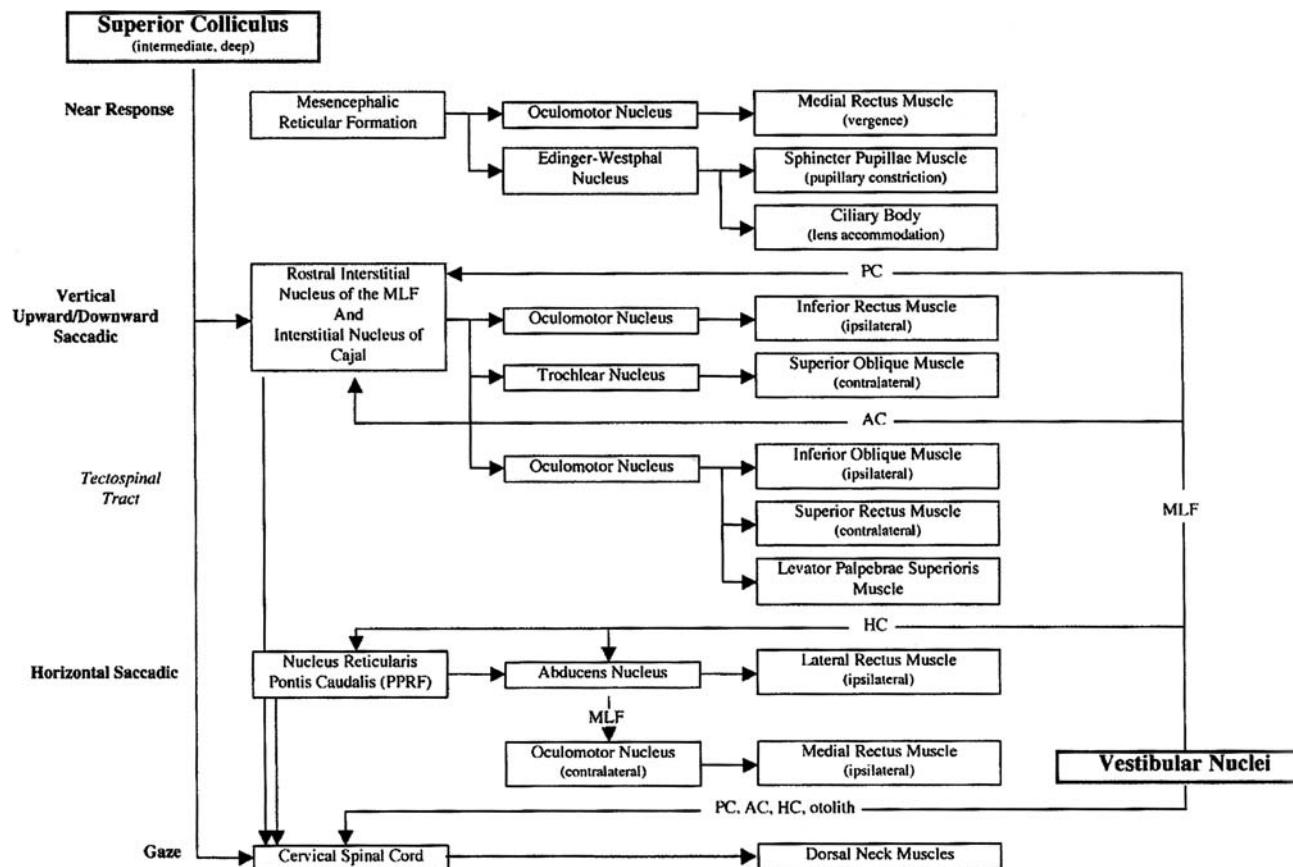


Fig. 28. Flow diagram of the major efferent connections of the superior colliculus and the vestibular nuclei that are related to the control of gaze. Connections related to accommodative vergence also are indicated. AC, anterior semicircular canal; HC, horizontal semicircular canal; MLF, medial longitudinal fasciculus; PC, posterior semicircular canal. Note how information from specific semicircular canals is distributed to the muscles that are appropriate for making the correct eye movements.

15. ROLE OF THE CEREBRAL CORTEX AND CEREBELLUM IN EYE MOVEMENT

Although many of the functions of the oculomotor system appear to involve mainly brain-stem structures, the cerebral cortex plays an important role in their proper operation. Despite the direct projections from the retina to the superior colliculus and pretectum, the binocularly and directional-selectivity features of the neuronal receptive fields in the superior colliculus and pretectum rely on descending connections from the primary and secondary visual areas of the cortex. Association areas of the cortex (e.g., frontal eye fields, occipitotemporal, and posterior parietal cortices) are largely responsible for the voluntary motor behaviors that are mediated by subcortical structures.

In contrast with the definitive role of the descending corticospinal and corticobulbar control from Brodmann's area 4 over spinal and other brain-stem

motor nuclei (e.g., trigeminal, facial, and hypoglossal), cortical motor control of eye movement, which originates from area 8 of the prefrontal cortex (e.g., "frontal eye fields"), is less direct. This cortical region has afferent cortico-cortical connections with visual cortical areas and is a major source of cortical input to the intermediate and deep layers of the superior colliculus. Lesions of this region produce only transient deficits in eye movements. The main deficit appears to be in eye movements that require attention to a particular stimulus in the visual field. Only combined lesions of the frontal eye field and the superior colliculus have a significant effect on voluntary eye movements, which is manifested by deficits in visually guided saccadic eye movements, particularly in the horizontal plane.

The *superior colliculus* is regarded as a site of sensorimotor transformation, and its function is related to orientation behavior. Inputs from any of the sensory systems can be converted into an eye

movement that is directed toward the stimulus. The superficial layers of the superior colliculus are associated predominately with visual inputs from the retina and the visual cortex. The deeper layers of the superior colliculus receive somatosensory, auditory, and visual inputs from a variety of cortical and subcortical areas (Fig. 29). The maps of the visual field, auditory space, and the body lie in register with one another and are superimposed on the motor map. This arrangement allows for stimulation of different points in the superior colliculus to produce saccades that differ in amplitude and direction. Thus, saccades are precisely directed to a target, regardless of whether that target is visual, auditory, or another sensory modality.

The output neurons in the deeper layers of the superior colliculus project by way of the *tectospinal tract* to brain-stem premotor areas in the mesencephalic and pontine reticular formation that control vertical and horizontal saccadic eye movements and to motor neurons in the cervical spinal cord that innervate the muscles of the neck (Fig. 28 and Fig. 29). The activity of these deep-layer efferent neurons encodes information about the direction, velocity, and amplitude of saccades.

Cortical projections to the *prectum*, (e.g., nucleus of the optic tract) and *accessory optic nuclei* are involved in the optokinetic reflex (Fig. 30). In the basic reflex, visual projections through prectum and accessory optic nuclei are relayed through the

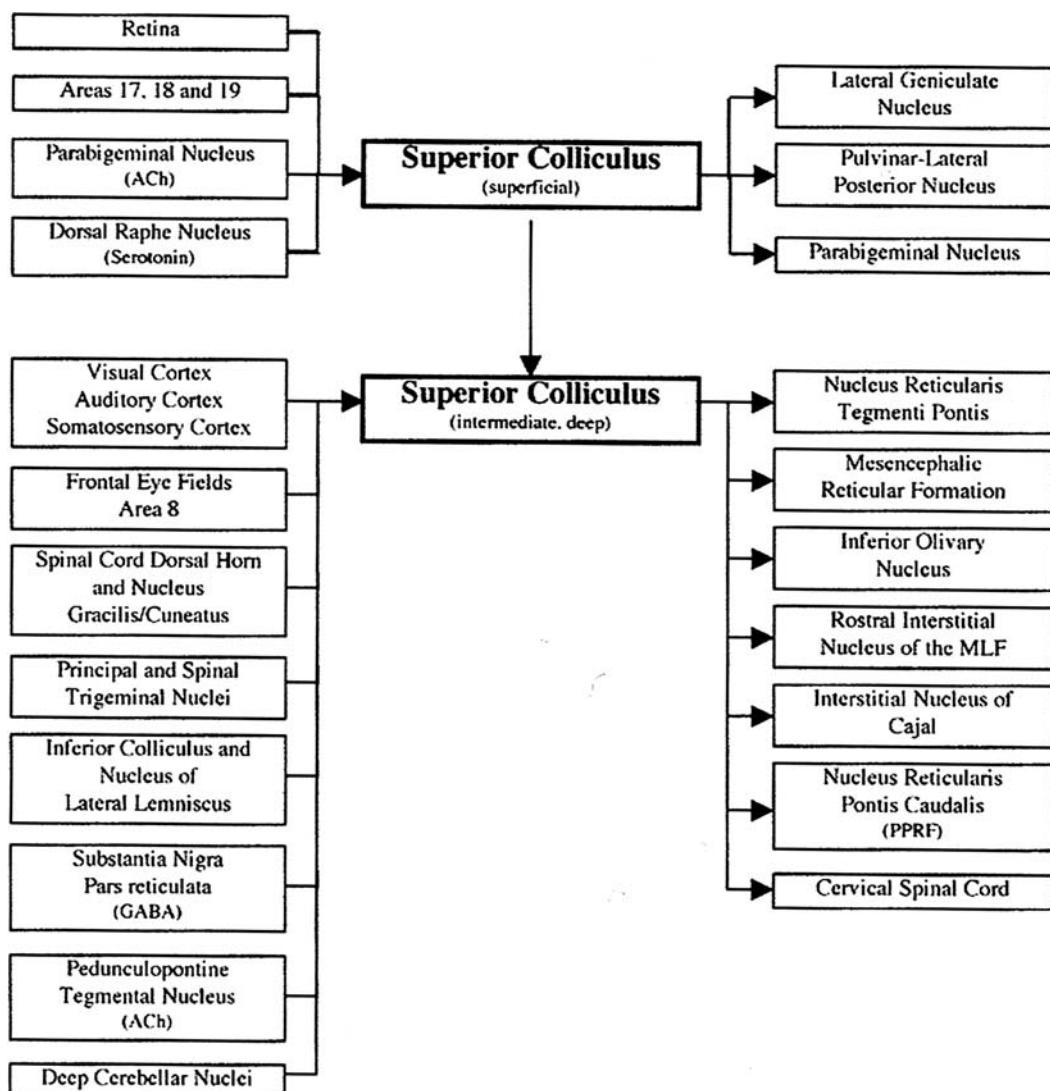


Fig. 29. Flow diagram of the major afferent and efferent connections of the superficial and deep layers of the superior colliculus. Ach, acetylcholine; GABA, gamma-aminobutyric acid; PPRF, paramedian pontine reticular formation.

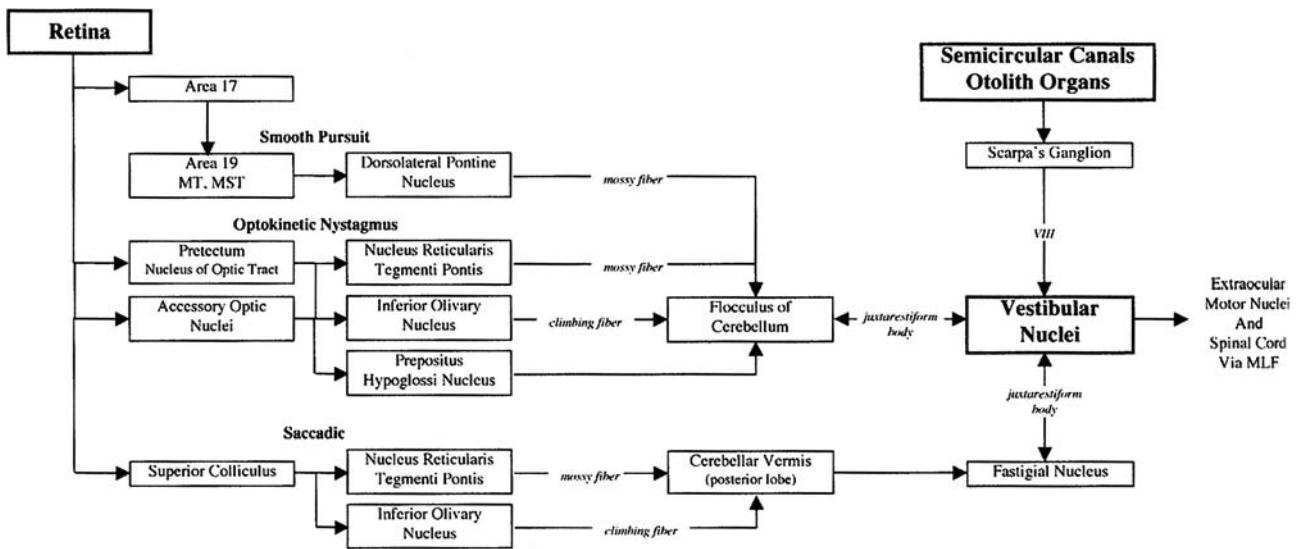


Fig. 30. Flow diagram of the structures and pathways related to the convergence of visual and vestibular information in the cerebellum and their role in smooth pursuit, optokinetic, and saccadic eye movements. The superior colliculus, pretectum, and accessory optic nuclei project to precerebellar relay nuclei in the basilar pons (nucleus reticularis segmenti pontis, dorsolateral pontine nucleus) and medulla (inferior olfactory nucleus). These regions project visual information to the same areas of the cerebellum that receive input from neurons in the vestibular ganglion and vestibular nuclei. The output of the cerebellum is directed to neurons in the vestibular nuclei that project to the extraocular motor nuclei and the spinal cord through the MLF.

nucleus reticularis segmenti pontis, the inferior olive, and the prepositus hypoglossi nucleus to the cerebellar flocculus. From there, movements generated by the optokinetic pathway converge upon the vestibular nucleus, where they share projections with the VOR, to motor neurons for the appropriate eye movements. Regions of the occipitotemporal and parieto-occipital cortex also appear to be important for the modulation of optokinetic responses, because lesions of the posterior parietal cortex can alter the bilateral, direction-specific response of this reflex.

The last class of smooth eye movements—smooth pursuit—also converges upon second-order vestibular neurons to share the same pathways for sending eye-position signals to motor neurons. Pursuit signals originate with visual inputs to cortical area 19, pass through pontine nuclei (the *dorsolateral pontine nucleus*) that are distinct from those used by the optokinetic reflex, pass through the cerebellar flocculus, and then to the vestibular nuclei, which in turn carry the pursuit signal to motor neurons (Fig. 30).

The cerebellum appears to play a key role in integrating sensory inputs from a variety of sources and using these to direct and recalibrate eye movements. Total cerebellectomy produces persistent deficits in smooth pursuit, optokinetic nystagmus, and holding eccentric positions of gaze. Moreover, loss of total cerebellar function interferes with the ability to use visual information to recalibrate eye movements after

paresis of one or more extraocular muscles. The vestibulocerebellum, which includes the *flocculus*, nodulus, and uvula, specifically controls eye movements that stabilize images on the retina whether the head is still (e.g., smooth pursuit) or moving (e.g., cancellation of the VOR). The *flocculus* of the cerebellum is the site of convergence of primary vestibular fibers and axons from brain-stem precerebellar nuclei, for example, nucleus reticularis segmenti pontis (via mossy fibers), dorsolateral pontine nucleus (via mossy fibers), and inferior olfactory nucleus (via climbing fibers). Because both optokinetic reflex and smooth pursuit are smooth eye movements that result from visual stimuli, their convergence at the flocculus allows the conservation of neurons into a common pathway that serves similar purposes (Fig. 30). The *fastigial nucleus* of the cerebellum is primarily related to the visual portion of the vermis of the posterior lobe, which also has afferent connections from the same brain-stem nuclei but to those regions that are synaptically related to the superior colliculus (Fig. 30). The dorsal cerebellar vermis and fastigial nuclei are important for the control of saccadic amplitude and accuracy. In summary, the cerebellum can be considered as the principal site of convergence of visual and vestibular information, which is transmitted to the oculomotor system by means of cerebellovestibular projections from the flocculus and fastigial nuclei to the vestibular nuclei.

16. ROLE OF THE BASAL GANGLIA IN EYE MOVEMENT

Diseases of the basal ganglia, including Parkinsonism and Huntington's chorea, are characterized by deficits in saccadic eye movements. The GABA-mediated inhibitory projection from the pars reticulata of the substantia nigra to the intermediate layer of the superior colliculus provides one pathway through which the basal ganglia may influence oculomotor control. This region of the substantia nigra receives input from the caudoputamen (e.g., striatum) and the subthalamus and forms part of an indirect corticotectal circuit by which sensory information from widespread regions of the cerebral cortex gain access to the superior colliculus. Neurons in the substantia nigra and the caudate nucleus discharge before intentional saccades.

Another link between the basal ganglia and the oculomotor system may be provided by an excitatory cholinergic projection to the superior colliculus, which arises from a collection of neurons in the caudal mesencephalic and rostral pontine tegmentum that partially corresponds with the pedunculopontine tegmentum nucleus or cholinergic-cell group Ch5. This region receives afferent connections from the pars reticulata of the substantia nigra and has efferent connections with the pars compacta of the substantia nigra and the subthalamus. This region of the tegmentum has been the only location from which the resting tremor that is the hallmark of Parkinson's disease has been produced by experimental lesions.

17. CONTROL OF VERGENCE EYE MOVEMENTS

Vergence eye movements consist of the coordinated contraction of medial rectus muscles bilaterally for convergence and coordinated contraction of the lateral rectus muscles bilaterally for divergence. Although these movements are in the horizontal plane, they do not use the MLF pathway, as they are not conjugate eye movements. The neural control of vergence eye movements is perhaps the least understood of all eye-movement systems. However, there is a loosely organized population of neurons that cap the oculomotor nucleus that discharge tonically in relationship to vergence angle—the angle formed between the two eyes as they converge or diverge. The cerebellum appears to play a key role in vergence eye movements, particularly in combined

conjugate-vergence movements. Neurons in parietal and visual association cortices discharge in relationship to vergence, but the precise anatomic pathways are not well-understood. Lesions of these cortical areas have been shown to compromise vergence.

18. BASIS OF DISEASE STATES

18.1. Anatomic Lesions at Various Points in the Visual Pathways Produce Characteristic Patterns of Visual Field Loss

The complexity of the visual system provides diagnostic clues as to the causes of at least some visual deficits. Lesions at different locations along the optic pathways result in characteristic visual defects (Fig. 31). Lesions of a single retina or optic nerve anterior to the optic chiasm result in a visual defect limited to one eye. A complete lesion of one optic nerve results in blindness of the corresponding eye. The pupil of the blind eye constricts consensually when light stimulates the opposite retina, because each pretectum receives input and sends output fibers bilaterally. However, direct stimulation of the blind eye in a darkened room reveals an unreactive dilated pupil. This leads to an afferent pupillary defect or Marcus-Gunn pupil. Lesions that occur beyond

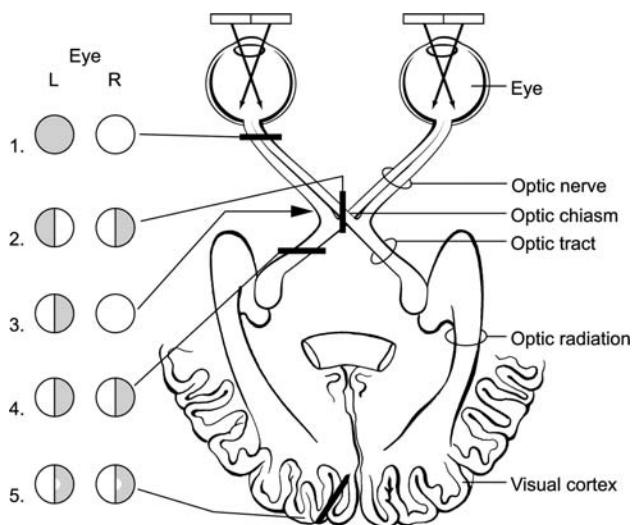


Fig. 31. Optic pathways in the brain and the visual field defects that their lesions produce: total visual loss of the left eye; bitemporal hemianopsia; left nasal hemianopsia; right homonymous (contralateral) hemianopsia; right homonymous hemianopsia with macular sparing. At the optic chiasm, nerve fibers from the nasal retina cross to the other side of the brain (decussate), joining the opposite optic nerve to form the optic tract.

the pretectum and superior colliculus do not affect the pupillary light reflex. Of course, bilateral lesions anterior to the chiasm affect both visual fields. This can occur symmetrically in diseases such as macular degeneration, which affects the macula, resulting in isolated central visual defects (called a central scotoma).

Lesions at or behind the optic chiasm give recognizable visual field deficits that affect both visual fields. Lesions that compress the optic chiasm (e.g., pituitary tumors) damage the crossing fibers preferentially, producing bitemporal hemianopia. Rarely, one or both lateral angles of the chiasm may be compressed, causing destruction of the noncrossing fibers and resultant nasal or binasal hemianopia.

Unilateral lesions of the optic pathways behind the chiasm (e.g., optic tract, lateral geniculate body, optic radiation, or visual cortex) result in a loss of the corresponding opposite field of vision, ranging from specific field defects to complete opposite-field loss, known as homonymous hemianopia. Occasionally, patterns of visual-field deficits can suggest a more precise localization of lesions along this pathway. The spatial relationships observed in the retina are maintained in the optic radiations. As the optic radiations sweep anteriorly and then posteriorly through the temporal lobe to form Meyer's loop, fibers from the inferior retina occupy the more anterior part of the path, and damage limited to this part of the optic radiation can cause a homonymous field defect limited to the superior quadrants (superior quadrantanopia). Conversely, injuries to the optic radiations in the parietal lobes can result in a homonymous field defect limited to the inferior quadrants (inferior quadrantanopia).

Occipital lesions often result from cerebrovascular accidents involving branches of the posterior cerebral artery. Because the tip of the occipital lobe, which is responsible for central vision, is supplied collaterally from the middle cerebral artery, central (e.g., macular) vision can be preserved, whereas the rest of the visual field is ablated. This causes a homonymous hemianopia with macular sparing.

Diseases of the visual system are not limited to gross morphologic damage. Many genetic disorders that affect vision have been described, affecting primarily the rods, cones, or both, but it is only with the advent of molecular biology that the molecular basis of these has been described. Two are considered here: blue cone monochromacy, which is a form of color blindness, and retinitis pigmentosa.

18.2. Loss of Color Vision Can Result from Genetic Lesions Causing Defects in the Color Pigments

An interesting ophthalmologic disease that has been elucidated at a molecular level is blue cone monochromacy (BCM). This is an X-linked form of color blindness in which only the short-wavelength-sensitive (*blue-sensitive*) cones are present. Clinically, it is characterized by poor visual acuity, nystagmus, glare-induced visual loss, and severely limited color vision in males. In some families, the retinas of affected individuals show progressive macular atrophy.

The highly similar red and green color pigment genes normally occur as a head-to-tail tandem array on the long arm of the X chromosome, with a red pigment gene followed by one or more highly similar green pigment genes. Most instances of BCM arise from unequal homologous recombination events that occur in this array during meiosis, resulting in the deletion of all but a single remaining gene (in some cases, a red-green hybrid gene). Alternatively, two genes may be present, one inactivated by a point mutation. Finally, deletions in a locus control region approximately 4 to 18 kilobases upstream from the red pigment gene can result in inactivation of both genes. These molecular lesions leave the patient able to discriminate brightness using rod input. However, they have essentially absent color vision, as only one of the three cone types, the blue cone pigment gene on chromosome 7, is present. Conversely, mutations in the blue cone pigment gene can lead to tritanopia, in which individuals lack blue and yellow discrimination while retaining red and green discrimination. The dominant inheritance of tritanopia, although incomplete, suggests that these mutations decrease either the viability or fidelity of blue-sensitive cone photoreceptors.

The elegant delineation of the pathophysiology of BCM and tritanopia by Jeremy Nathans and his collaborators is a classic example of the power of combining the techniques of molecular genetics with clinical acumen. Identification of the three color pigment genes and the clinical effects of mutations in them essentially proved the trichromatic theory of human color vision first put forth by Thomas Young in 1802.

18.3. Retinitis Pigmentosa Can Result from Mutations in Highly Expressed Retinal-Specific Genes

Retinitis pigmentosa (RP) is the clinical term used to describe a large, genetically and phenotypically heterogeneous group of visual disorders in which the rod photoreceptors degenerate, resulting in

partial or total blindness. These are characterized by poor rod function early on and a progressive degeneration of the retina that begins at the midperiphery. RP has a variable age of onset and rate of progression and affects approximately 1 in every 2000 to 4000 persons worldwide.

For the purposes of clinical diagnosis, the classic symptoms of RP include night blindness (e.g., nyctalopia) early in the disease, alterations in the ERG (Fig. 32), and the deposit of so-called bone spicules of pigment on the retinal surface, accounting for the name of the disease. The early onset of night blindness and narrowing of the visual fields are consistent with the rods being affected first in RP, as they are sensitive to low levels of light and are found in the periphery of the retina, whereas the cones are primarily responsible for color and detail vision and are concentrated in the macula. RP shows genetic heterogeneity as well. Autosomal dominant (adRP), autosomal recessive, and X-linked forms of RP have been described.

Lesions in multiple genes have been shown to cause RP. In 1989, Peter Humphries and colleagues established the linkage between one form of adRP and a marker on the long arm of chromosome 3 in a large Irish pedigree. The gene for human rod opsin had been previously shown to map to the long arm of

chromosome 3. Using this knowledge, Dryja and his colleagues quickly identified a point mutation in the rhodopsin gene that could be identified as the defect in 17 of 148 unrelated adRP families. The specific change, a C to A transversion, resulted in the substitution of a histidine for a highly conserved proline at amino acid 23, in the amino-terminal region of the mature opsin moiety. In a mouse line that was transgenic for the P23H rhodopsin mutation (along with two others) the photoreceptors developed normally, but the light-sensitive outer segments never reached normal length. As the mice aged, their retinas showed a slow but progressive retinal degeneration with decreased light-evoked responses on ERG. Many other rhodopsin mutations have been described in patients with RP, accounting for about 25% of all RP cases. Identification of additional genes associated with RP has been carried out by a combination of linkage analysis and by systematically screening candidate genes for mutations in individuals who are affected by RP.

Currently, approximately 190 genes that cause inherited retinal diseases when mutated have been mapped in the human genome, and more than two-thirds of these have been identified and cloned and the causative mutations characterized. The number of these genes is growing weekly, so that an updated

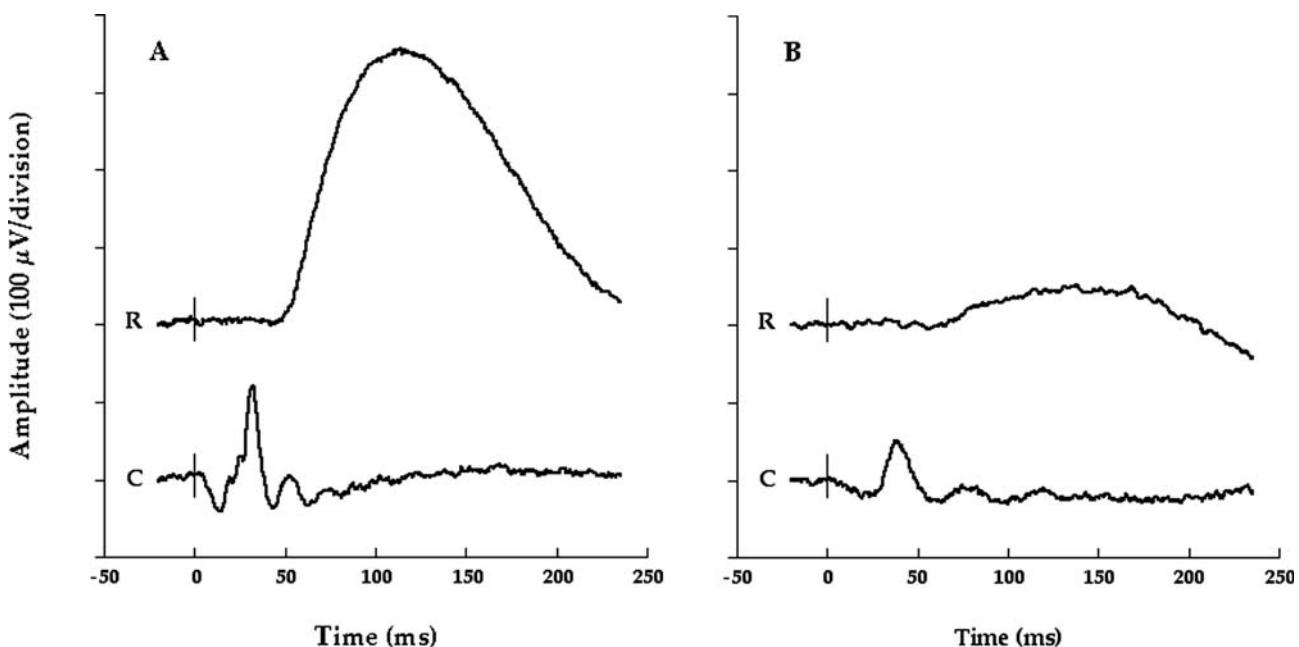


Fig. 32. (A) Normal human electroretinogram (ERG). R, rod-mediated response elicited by a dim flash stimulus on a dark-adapted retina; C, cone-mediated response elicited by a bright flash stimulus on a light-adapted retina. **(B)** ERG of patient with early retinitis pigmentosa (RP). Note that the rod-mediated response (R) is markedly reduced, whereas the cone-mediated response (C) is less severely compromised.

summary is maintained on the RetNet Web site. Sixteen genes have been implicated in autosomal dominant RP, of which 14 have been cloned. Eighteen additional genes have been implicated in autosomal recessive RP, of which 13 have been cloned, and 6 genes have been implicated in X-linked RP, with 2 cloned. Current studies are examining the precise molecular mechanisms whereby the mutant opsins give rise to the RP phenotype. Preliminary studies of some alleles indicate that these defective opsins are poorly transported to and incorporated into the membrane, and others result in a constitutively activated phototransduction cascade. However, the detailed pathophysiology leading to the relatively late onset of symptomatic RP has yet to be delineated. It seems likely that mutations in various photoreceptor-specific genes can have a direct effect on vision through changes in their function and perhaps cause RP by damaging retinal cells sufficiently to induce apoptosis as a common final pathway of cellular death.

These candidate genes often encode phototransduction proteins or other proteins produced at high levels in the retina. In addition to rhodopsin, perhaps the most prominent retinal protein, mutations in peripherin, which stabilizes the disks in the rod outer-segment membranous disks, cause RP. Other proteins that have been implicated in RP include rod cGMP-gated channel alpha subunit, arrestin, the alpha and beta subunits of cGMP phosphodiesterase, RPE-retinal GPCR, cellular retinaldehyde-binding protein, cyclic nucleotide-gated channel alpha-1 (CNGA1), and rod outer-segment membrane protein 1. Retinal degenerations are caused by mutations in a wide variety of genes encoding proteins ranging from transcription factors such as neural retina leucine zipper (NRL) to pre-mRNA processing factor 3 homolog (PRPF3). Interestingly, many of the genes associated with RP can, when mutated at a different amino acid or in some cases even with the same mutation, be inherited as either dominant or recessive traits, or even cause other retinal degenerations such as congenital stationary night blindness (rhodopsin), macular degeneration (peripherin), and others.

Mutations in rhodopsin, peripherin, and the other implicated proteins still account for a minority of RP cases, indicating that many more genes may be involved in other forms of the disorder. Probable candidates include genes encoding proteins involved in phototransduction specifically and genes that code for retinal housekeeping-type proteins. Several laboratories are actively looking for the genes and for lesions that may be linked to RP.

The clinical heterogeneity of RP, even among siblings carrying the same RP-causing mutation, is still not explained, although a variety of factors such as modifying genes and environmental differences can be invoked. Eventually, it is possible that such studies will lead to cures for selected cases of RP-induced blindness.

19. DISORDERS OF OCULAR MOTILITY

Coordinated eye movements occur via saccades, smooth pursuit, the vestibular ocular reflex, and convergence. These movements depend on intact supranuclear control mechanisms, brain-stem gaze centers, certain cranial nerves, extraocular muscles, and neuromuscular transmission. Dysfunction of any of these components impairs ocular motility and produces a variety of distinct visual and neurologic signs and symptoms.

19.1. Causes of Eye-Movement Disorders

19.1.1. SEVERAL SUPRANUCLEAR SYSTEMS GUIDE EYE MOVEMENT

The saccadic system allows visual refixation on an object of interest seen in the periphery by moving the eyes rapidly until the image of the object is moved onto the fovea and into central vision. Saccades may be either intentional or reflexive. The pursuit system moves the eyes at the appropriate speed to hold the image of a slowly moving object on the fovea. The vergence system allows the eyes to move apart or toward one another so that images of objects at various distances can be kept on the fovea of both eyes simultaneously. The vestibular system moves the eyes to compensate for head movements so that images remain on the fovea during such activity.

Abnormalities of saccadic eye movement can result from lesions of the frontal eye fields, supplemental eye fields, parietal eye fields, pontine and mesencephalic reticular nuclei, the cerebellum, or the basal ganglia, which constitute some of the major structures of the system responsible for the generation of this form of ocular movement. Acute lesions of the eye fields in the frontal lobe result in the inability to voluntarily initiate a saccade in the direction opposite the lesion. In a patient with a stroke involving the right frontal lobe, the eyes are deviated to the right and cannot voluntarily be moved to the left beyond the midline. However, by evoking reflex movements of the eyes, they can be moved to the left, indicating that the cranial nerve nuclei, cranial

nerves, and extraocular muscles used for movement in this direction are still intact. This form of eye-movement disturbance in the face of intact cranial nerves and nuclei is known as a supranuclear gaze palsy. Another way in which saccadic movements can be abnormal is in relationship to their speed. In certain degenerative conditions of the cerebellum or basal ganglia, saccades become slowed. In Huntington's disease, for example, the appearance of slow saccades may be one of the earliest signs of neurologic dysfunction.

Like the saccadic system, the pursuit system incorporates several CNS structures, including the striate and extrastriate cortex, pontine nuclei, vestibular nuclei, and the cerebellum. Lesions in a variety of anatomic sites may interfere with pursuit movement. When pursuit fails, the speed of eye movement does not keep pace with a moving target. To compensate, corrective saccades are periodically generated, giving the otherwise smooth eye movements a jerky or ratchet-like character. These jerky movements are known as saccadic pursuit. A lesion of the parieto-temporal cortex on one side can impair pursuit, resulting in saccadic pursuit when following objects moving toward the side of the lesion. The ipsilateral nature of this impairment has been attributed to a double decussation of the brain-stem pathways. After cortical projections reach pontine nuclei, there are decussations between these pontine nuclei and the cerebellum and then between the vestibular nucleus, and the abducens nucleus.

19.1.2. ABNORMALITIES OF CONJUGATE GAZE IMPLY BRAIN STEM

When both eyes move in the same direction at the same speed, maintaining a constant alignment, gaze is said to be conjugate. The center controlling horizontal conjugate gaze is located in the pons in the vicinity of the abducens nucleus and is known as the paramedian zone of the pontine reticular formation. A lesion involving the pontine pararectal formation on one side results in inability to move either eye beyond the midline toward that side. Unlike supranuclear gaze paralysis caused by frontal lobe lesions, this form of gaze paresis cannot be overcome by inducing involuntary reflex eye movements. The vertical conjugate gaze center is located in the midbrain. Lesions in this region, such as a tumor of the pineal gland compressing the dorsal midbrain, produce paralysis of conjugate vertical gaze, which in this case is an inability to gaze upward.

The cranial-nerve nuclei on both sides of the brain stem subserving conjugate eye movements are connected by the medial longitudinal fasciculus (MLF) fiber tract. An isolated lesion of this tract produces a specific abnormality of ocular motility. Because the cranial-nerve nuclei are left intact by a MLF lesion, eye movements in all directions are still possible, but because the nuclei are disconnected, the eyes do not move in a conjugate manner in the horizontal plane. When left lateral gaze is attempted by an individual with a right MLF lesion, the right eye, which must move inward, cannot cross the midline, although the outward-moving left eye responds normally. The same eye that could not move inward during attempted voluntary lateral gaze can do so when both eyes converge on a near target, proving the intactness of the third cranial nerve and medial rectus muscles underlying this movement. This pattern of deficient inward eye movement resulting from a MLF lesion is referred to as an *internuclear ophthalmoplegia*. This abnormality can be caused by any lesion interrupting the MLF, but multiple sclerosis is the most common cause, especially when the MLF is affected on both sides.

19.1.3. DISTINCT PATTERNS OF IMPAIRED OCULAR MOTILITY RESULT FROM LESIONS OF INDIVIDUAL CRANIAL NERVES

Abnormalities of the third (oculomotor), fourth (trochlear), or sixth (abducens) cranial nerves produce distinct patterns of impaired ocular motility. In an oculomotor-nerve palsy, the superior, inferior, and medial recti are paralyzed, as is the inferior oblique muscle. The affected eye deviates downward and laterally. This position results from the action of the two functioning ocular muscles, the lateral rectus and the inferior oblique, which are innervated by the abducens and trochlear nerves, respectively. In a complete oculomotor nerve palsy, the pupil is dilated and the eyelid droops. The patient complains of double vision (diplopia) in several directions of gaze because of the number of different muscles paralyzed. The oculomotor nerve can be damaged by ischemia in diabetes, be impinged on by an arterial aneurysm on the surface of the brain, or be compressed by brain tissue being forced downward by an expanding mass such as a tumor within the cranium. The latter turn of events often signals imminent death from compression of other vital brain structures if the causative mass effect is not corrected.

In an abducens nerve palsy, only the lateral rectus is weakened. The affected eye deviates medially, and

the globe cannot be moved laterally beyond the midline. The patient complains of double vision that is worse during lateral gaze toward the side of the lesion. The abducens nerve pursues a long intracranial course and is angled over bony structures at the base of the skull. Because it pursues this path, the nerve is susceptible to stretch after any displacement of the brain stem to which it is attached. An abducens-nerve palsy may appear in patients with increased intracranial pressure of any cause if the pressure results in slight downward displacement of the brain stem or after blunt trauma to the skull, for the same reason.

In a trochlear-nerve palsy, the superior oblique muscle is weak, resulting in inability to depress the globe, especially in the adducted (e.g., medial) position. In this syndrome, diplopia may occur, which is improved by tilting the head in the direction of the normal eye. The presence of head tilt, especially in a child, is often the first indication of the presence of a trochlear nerve-palsy.

19.1.4. DISORDERS OF MUSCLE OR NEUROMUSCULAR TRANSMISSION CAN AFFECT OCULAR MOTILITY

Ocular motility is involved in some disorders of muscle. This is especially true of the muscle disorder associated with thyroid disease and that seen in the mitochondrial disorders. In these two conditions, the degree of weakness of ocular muscles may be much greater than that seen in appendicular or truncal musculature.

Abnormalities of ocular motility are extremely common and are often the presenting sign in myasthenia gravis, a disorder of neuromuscular transmission. In this condition, any one or all of the ocular muscles can be involved. Most commonly, there is associated weakness and drooping of the eyelid. Myasthenia is characterized by abnormal fatigability, and unlike motility disturbances related to central nervous system disorders, the degree of motility impairment may vary from day to day and hour to hour, depending on the use of the eyes. For example, patients with myasthenia gravis often notice diplopia only after sustained gaze in the same direction, which may occur while watching TV or looking at an object in the sky. When impaired ocular motility and eyelid weakness appear in a myasthenic individual, a diagnosis of oculomotor-nerve palsy may be mistakenly made, but the absence of pupillary abnormality and the response to cholinesterase-inhibiting medications clearly establish myasthenia as the cause.

19.1.5. NYSTAGMUS IS AN ABNORMAL PATTERN OF REPETITIVE EYE MOVEMENTS

Nystagmus is a rhythmic to-and-fro oscillation of the eyes. It can occur in the vertical or horizontal plane and sometimes can be rotatory. It is usually phasic; the oscillation in one direction is faster than in the other direction. In pathologic states, nystagmus may occur when the eyes are in the primary position or after they are moved to their limit in one direction. *Gaze-evoked nystagmus* is usually seen in patients receiving sedative drugs and also occurs in cerebellar diseases. It occurs when gaze is directed away from the middle position in the vertical or the horizontal plane. *Downbeat nystagmus*, evident when the eyes are in the primary position, is most commonly caused by lesions in the vicinity of the craniocervical junction.

Vestibular nystagmus results from dysfunction of the vestibular apparatus or vestibular nerve. If the dysfunction is unilateral, the nystagmus has its fast phase directed away from the side of the lesion. There is often a rotatory component to the nystagmoid movement. Vertigo, a spontaneous hallucination of movement (often a sense of spinning), commonly accompanies vestibular nystagmus. Vestibular nystagmus can result from causes as diverse as a tumor of the eighth cranial nerve or viral labyrinthitis. Vestibular nystagmus can be readily provoked in normal persons by instilling ice water into the ear canal. This caloric stimulation sets up convection currents in the endolymph within the semicircular canals, resulting in nystagmus toward the side of stimulation and a profound sense of vertigo. This procedure can be used as a test of the intactness of the vestibular system.

Pendular nystagmus differs from most other types in that it is not phasic; the speed of the nystagmoid movement is equal in both directions. It is often congenital, in which case it may be associated with poor vision. If pendular nystagmus is acquired, it usually indicates brain-stem pathology, usually related to multiple sclerosis or stroke. *Physiologic nystagmus* appears at the extremes of gaze in normal persons, especially when the eye muscles are fatigued or when the eyes are held at the extreme of lateral or vertical gaze for an extended period. Approximately 5% of the population is capable of producing *voluntary nystagmus*, consisting of a 10- to 25-s burst of extremely rapid back-and-forth horizontal movements.

Acknowledgments This chapter incorporates, with minimal changes, material from the chapters "Critical Periods in Visual System

Development" authored by Dr. Mark F. Bear, "The Oculomotor System" by Dr. Robert F. Spencer and Dr. John D. Porter, and "Disorders of Ocular Motility" by Dr. Gregory Cooper and Dr. Robert L. Rodnitzky that appeared in the first edition of this work.

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Disorders of Ocular Motility

Gregory Cooper, Gerald Eichhorn and Robert Rodnitzky

Coordinated eye movements occur via saccades, smooth pursuit, the vestibular ocular reflex, and convergence. These movements depend on intact supranuclear control mechanisms, brain-stem gaze centers, certain cranial nerves, extraocular muscles, and neuromuscular transmission. Dysfunction of any of these components impairs ocular motility and produces a variety of distinct visual and neurologic signs and symptoms.

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Like the saccadic system, the pursuit system incorporates several CNS structures, including the striate and extrastriate cortex, pontine nuclei, vestibular nuclei, and the cerebellum. Lesions in a variety of anatomic sites may interfere with pursuit movement. When pursuit fails, the speed of eye movement does not keep pace with a moving target. To compensate, corrective saccades are periodically generated, giving the otherwise smooth eye movements a jerky or ratchet-like character. These jerky movements are known as saccadic pursuit. A lesion of the parieto-temporal cortex on one side can impair pursuit, resulting in saccadic pursuit when following objects moving toward the side of the lesion. The ipsilateral nature of this impairment has been attributed to a double decussation of the brain-stem pathways. After cortical projections reach pontine nuclei, there are decussations between these pontine nuclei and the cerebellum and then between the vestibular nucleus and the abducens nucleus.

ABNORMALITIES OF CONJUGATE GAZE IMPLY BRAIN-STEM PATHOLOGY

When both eyes move in the same direction at the same speed, maintaining a constant alignment, gaze is said to be conjugate. The center controlling horizontal conjugate gaze is located in the pons in the vicinity of the abducens nucleus and is known as the paramedian zone of the pontine reticular formation. A lesion involving the pontine parareticular formation on one side results in inability to move either eye beyond the midline toward that side. Unlike supranuclear gaze paralysis caused by frontal lobe lesions, this form of gaze paresis cannot be overcome by inducing involuntary reflex eye movements. The vertical conjugate gaze center is located in the midbrain. Lesions in this region, such as a tumor of the pineal gland compressing the dorsal midbrain, produce paralysis of conjugate vertical gaze, which in this case is an inability to gaze upward.

The cranial nerve nuclei on both sides of the brain stem subserving conjugate eye movements are connected by the medial longitudinal fasciculus (MLF) fiber tract. An isolated lesion of this tract produces a specific abnormality of ocular motility. Because the cranial nerve nuclei are left intact by a MLF lesion, eye movements in all directions are still possible, but because the nuclei are disconnected, the eyes do not move in a conjugate manner in the horizontal plane. When left lateral gaze is attempted by an individual with a right MLF lesion, the right eye, which must move inward, cannot cross the midline, although the outward-moving left eye responds normally. The same eye that could not move inward during attempted voluntary lateral gaze can do so when both eyes converge on a near target, proving the intactness of the third cranial nerve and medial rectus muscles underlying this movement. This pattern of deficient inward eye movement resulting from a MLF lesion is referred to as an internuclear ophthalmoplegia. This abnormality can be caused by any lesion interrupting the MLF, but multiple sclerosis is the most common cause, especially when the MLF is affected on both sides.

DISTINCT PATTERNS OF IMPAIRED OCULAR MOTILITY RESULT FROM LESIONS OF INDIVIDUAL CRANIAL NERVES

Abnormalities of the third (oculomotor), fourth (trochlear), or sixth (abducens) cranial nerves produce distinct patterns of impaired ocular motility. In

oculomotor nerve palsy, the superior, inferior, and medial recti are paralyzed, as is the inferior oblique muscle. The affected eye deviates downward and laterally. This position results from the action of the two functioning ocular muscles, the lateral rectus and the inferior oblique, which are innervated by the abducens and trochlear nerve, respectively. In complete oculomotor nerve palsy, the pupil is dilated and the eyelid droops. The patient complains of double vision (diplopia) in several directions of gaze because of the number of different muscles paralyzed. The oculomotor nerve can be damaged by ischemia in diabetes, compressed by an arterial aneurysm on the surface of the brain or by brain tissue being forced downward by an expanding mass such as a tumor within the cranium. The latter turn of events often signals imminent death from compression of other vital brain structures if the causative mass effect is not corrected.

In abducens nerve palsy, only the lateral rectus is weakened. The affected eye deviates medially, and the globe cannot be moved laterally beyond the midline. The patient complains of double vision that is worse during lateral gaze toward the side of the lesion. The abducens nerve pursues a long intracranial course and is angled over bony structures at the base of the skull. Because it pursues this path, the nerve is susceptible to stretch after any displacement of the brain stem to which it is attached. Abducens nerve palsy may appear in patients with increased intracranial pressure of any cause if the pressure results in slight downward displacement of the brain stem or after blunt trauma to the skull.

In a trochlear nerve palsy, the superior oblique muscle is weak, resulting in inability to depress the globe, especially in the adducted (e.g., medial) position. In this syndrome, diplopia may occur, which is improved by tilting the head in the direction of the normal eye. The presence of head tilt, especially in a child, is often the first indication of the presence of a trochlear nerve palsy.

DISORDERS OF MUSCLE OR NEUROMUSCULAR TRANSMISSION CAN AFFECT OCULAR MOTILITY

Ocular motility is involved in some disorders of muscle. This is especially true of the muscle disorder associated with thyroid disease and that seen in mitochondrial disorders. In these two conditions, the degree of weakness of ocular muscles may be much greater than that seen in appendicular or truncal musculature.

Abnormalities of ocular motility are extremely common and often the presenting sign in myasthenia gravis, a disorder of neuromuscular transmission. In this condition, any one or all of the ocular muscles can be involved. Most commonly, there is associated weakness and drooping of the eyelid. Myasthenia is characterized by abnormal fatigability, and unlike motility disturbances related to central nervous system disorders, the degree of motility impairment may vary from day to day and hour to hour, depending on the use of the eyes. For example, patients with myasthenia gravis often notice diplopia only after sustained gaze in the same direction, which may occur while watching TV or looking at an object in the sky. When impaired ocular motility and eyelid weakness appear in a myasthenic patient, a diagnosis of oculomotor nerve palsy may be mistakenly made, but the absence of pupillary abnormality and the response to cholinesterase-inhibiting medications clearly establish myasthenia as the cause.

NYSTAGMUS IS AN ABNORMAL PATTERN OF REPETITIVE EYE MOVEMENTS

Nystagmus is a rhythmic to-and-fro oscillation of the eyes. It can occur in the vertical or horizontal plane and sometimes can be rotatory. It is usually phasic; the oscillation in one direction is faster than in the other direction. In pathologic states, nystagmus may occur when the eyes are in the primary position or after they are moved to their limit in one direction. Gaze-evoked nystagmus is usually seen in patients receiving sedative drugs and also occurs in cerebellar diseases. It occurs when gaze is directed away from the middle position in the vertical or the horizontal plane. Downbeat nystagmus, evident when the eyes are in the primary position, is most commonly caused by lesions in the vicinity of the craniocervical junction.

Vestibular nystagmus results from dysfunction of the vestibular apparatus or vestibular nerve. If the dysfunction is unilateral, the nystagmus has its fast phase directed away from the side of the lesion. There is often a rotatory component to the nystagmus. Vertigo, a spontaneous illusion of movement, commonly accompanies vestibular nystagmus. Vestibular nystagmus can result from causes as diverse as a tumor of the eighth cranial nerve or viral labyrinthitis. Vestibular nystagmus can be readily provoked in normal persons by instilling ice water into the ear canal. This caloric stimulation sets up convection currents in the endolymph within the semicircular canals, resulting in nystagmus toward the side of stimulation and a profound sense of vertigo. This procedure can be used as a test of the intactness of the vestibular system.

Pendular nystagmus differs from most other types in that it is not phasic; the speed of the nystagmoid movement is equal in both directions. It is often congenital, in which case it may be associated with poor vision. If pendular nystagmus is acquired, it usually indicates brain-stem pathology, most often related to multiple sclerosis or stroke. Physiologic nystagmus appears at the extremes of gaze in normal persons, especially when the eye muscles are fatigued or when the eyes are held at the extreme of lateral or vertical gaze for an extended period. Approximately 5% of the population is capable of producing voluntary nystagmus, consisting of a 10- to 25-s burst of extremely rapid back-and-forth horizontal movements.

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Tom C.T. Yin

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1. INTRODUCTION

This chapter provides an introduction to the physiological mechanisms underlying auditory perception. We first consider the physical properties of sound, then examine the processing of acoustic input by the peripheral auditory system followed by the central auditory system.

2. THE PHYSICAL NATURE OF SOUND

A few basic properties of sound must be understood before analyzing the auditory system. Physically, sound is a mechanical disturbance that is propagated through an elastic medium. This medium usually is air, but sound can also propagate through solids or liquids. Air molecules are in constant, random motion such that the large number of air molecules striking any given point in space produces a static pressure, which depends on conditions of the system, such as the density of gas and air temperature. With a sudden change in the position of some large object in the air, such as clapping hands or vibrating the cone of a loudspeaker, the mechanical disturbance causes a temporary increase in pressure locally with a corresponding decrease elsewhere (e.g., increase on one side of the speaker cone and decrease

on the other). The disturbance is propagated through the medium as the air molecules collide with each other and transfer energy to neighboring molecules. This variation in pressure as a function of time is called a *sound wave*. It represents the characteristics of the population of molecules as a whole rather than any single air molecule, which moves back and forth randomly without necessarily propagating energy.

The velocity at which the wave travels depends on the density and elasticity of the medium. Sound travels about four times faster in water than in air. The strength of the sound wave is usually measured as the deviation in sound pressure from atmospheric pressure.

Most sound waves result from the vibrations of an object, and the simplest form of vibration is a sine wave, which produces a pure tone. Figure 1 shows a diagram of the instantaneous pressure and the spatial distribution of air molecules as a function of distance in the medium for a pure tone at one instant in time. It can be considered to be a snapshot of the sound wave at some moment. The wavelength of the sound is the distance between successive peaks. Because sound propagates through air at a constant velocity (340 m/s or taking about 30 μ s to travel 1 cm), the abscissa in Fig. 1 could also be time; it would then depict the variation in pressure at a given point in the medium as a function of time. In this case the distance between peaks would be the *period* of the tone.

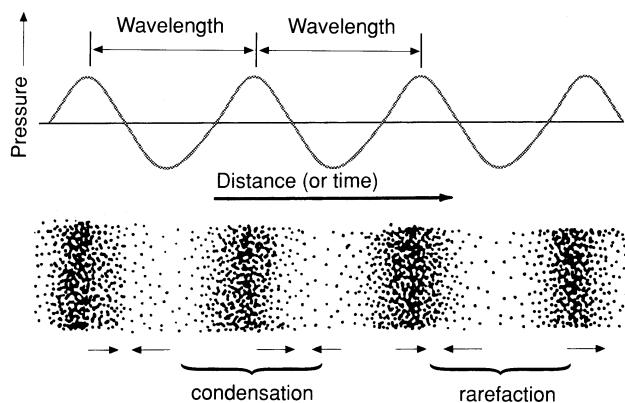


Fig. 1. Diagram of air molecules in response to sinusoidal sound vibrations. The rarefactions and condensations are shown.

The relation between the wavelength λ , conduction velocity c , frequency f expressed in cycles/second or hertz (Hz), and the period T of the sine wave is given by $\lambda = c/f$, in which $f = 1/T$. For a 100-Hz pure tone, which is near the lower limit of human hearing, λ is 3.3 m; at 10,000 Hz, it is 3 cm. The musical note middle C has a frequency of 256 Hz. These variations in wavelength with frequency are important in discussing the cues that are available for sound localization.

The sensitivity of the auditory system is quite remarkable. On the lower end, the faintest sound that can be detected by humans is generated by movements of the eardrum of approximately 10^{-10} m, which is in the range of diameters of air molecules. On the high end, sound pressures that are 10^6 larger begin to result in painful sensations. Because of this large dynamic range, the intensities of sounds are usually expressed in decibels (dB), which is a logarithmic unit of ratios. The arithmetic definition of a decibel is $\text{dB} = 20 \log_{10}(P/P_{\text{ref}})$, in which P is the pressure of interest and P_{ref} is a reference pressure, which can be arbitrarily chosen. Usually, P_{ref} is chosen to be near the average normal threshold of hearing, and, when so chosen, the decibels are denoted as dB sound pressure level (SPL). The dynamic range in humans is about $20 \log_{10} 10^6 = 120$ dB.

The physical parameters of frequency and SPL correspond with the perceptual qualities of *pitch* and *loudness*, respectively. In audiology and speech analysis, any particular sound can be represented by a spectrogram that plots the SPL as the darkness of spots on a time-frequency axis, as shown in Fig. 2. A pure tone is represented by a single frequency that persists over time, a musical note generally has many harmonics, and speech consists of a complex mixture

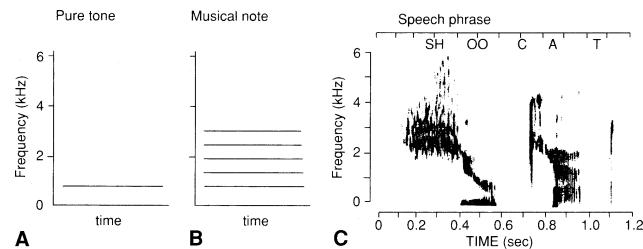


Fig. 2. Spectograms of (A) a pure tone of 800 Hz, (B) a musical note from a flute, and (C) a speech phrase (i.e., "shoo cat"). Darkness represents acoustic energy in the signal at the corresponding frequency. (Adapted from Kiang NYS. Stimulus representation in the discharge patterns of auditory neurons. In Tower DB (ed.). The Nervous System, Vol. 3: Human Communication and Its Disorders. New York: Raven Press, 1975:81.)

of frequencies varying with time. A topic of considerable clinical interest is how the auditory system can encode a complex sound, such as speech, and distinguish the subtle differences between similar sounds or the same sound uttered by different people that constitute daily human experience. We know very little about these questions.

3. THE PERIPHERAL AUDITORY SYSTEM

It is convenient to divide the ear into an outer, middle, and inner ear, as shown in Fig. 3, which depicts a cross section of the human ear. A standard sequence of events leads to activation of auditory nerve fibers in response to an acoustic stimulus. Sound waves enter the external ear and travel down the external auditory meatus to strike the tympanic membrane, or eardrum. Movements of the tympanic membrane are transferred by means of the ossicular chain to the oval window of the cochlea, and back and forth movements of the oval window cause a traveling wave to be set up in the fluid-filled cochlear ducts. The traveling wave causes deflections of the basilar membrane, which vibrates and activates the receptor cells, the hair cells in the organ of Corti, which in turn activate auditory nerve fibers that carry the information into the brain.

3.1. The Outer Ear Is Important for Collecting Sound Waves

The outer ear consists of the pinna, or external ear, and the external auditory meatus, or ear canal. This apparatus has important acoustic properties, because it accentuates or attenuates sounds of certain frequencies and those coming from particular

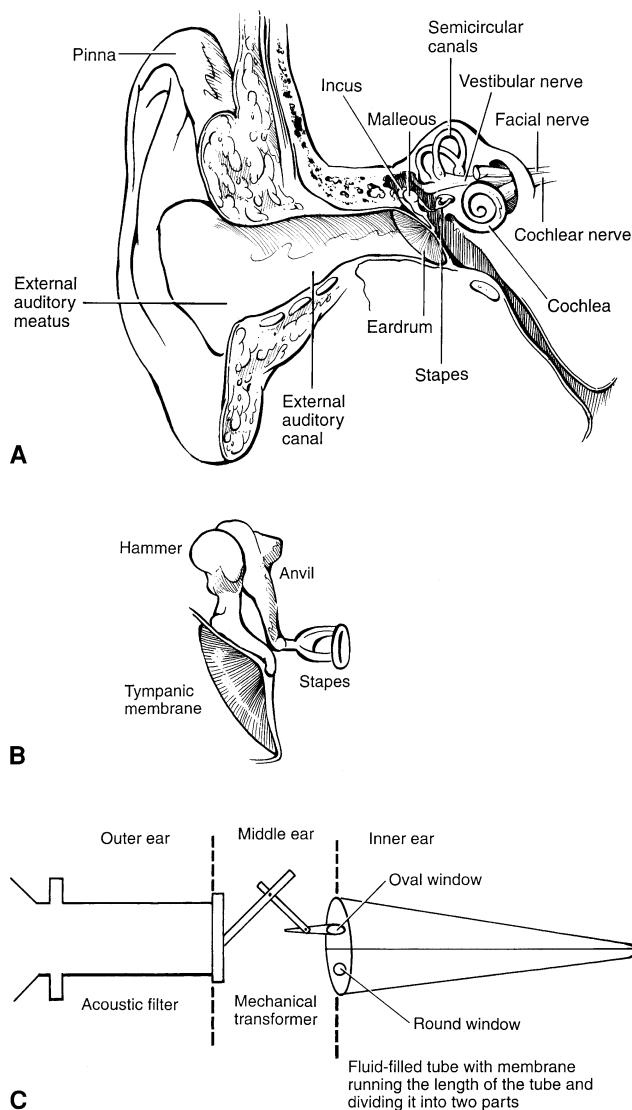


Fig. 3. Major divisions of the peripheral auditory system. **(A)** The outer, middle, and inner ear. **(B)** An enlarged version of the middle ear. **(C)** The mechanical analogues of the outer and middle ear.

directions. The degree to which the pinna can influence a sound wave depends on the wavelength of the sound and hence its frequency. If the wavelength is much longer than the dimensions of the pinna, as it is for low-frequency sounds, the pinna appears transparent to the sound and produces no diffractive or reflective effects. Only high-frequency sounds of at least 5 kHz are greatly affected by the pinna. Because of the directional properties of the pinna, these transformations are important for localizing sounds, particularly those in the vertical plane. In humans, unlike most other mammals, there is virtually no control over the direction in which the pinna is pointing other than movements of the head.

3.2. The Middle Ear Acts as an Impedance Matching Device

The middle ear consists of the *tympanic membrane* and the three bony *ossicles* that conduct vibrations to the oval window: the *malleus*, *incus*, and *stapes* (i.e., hammer, anvil, and stirrup). The middle ear is important in providing an *impedance matching*, or interface, for the airborne sound waves to transmit their energy to the fluid in the cochlea. If there were no middle ear, only 0.1% of the energy of a sound wave would be transmitted to the fluid in the cochlea. The middle ear reduces this energy loss, chiefly by the mechanical advantage resulting from the reduction in surface area from the large tympanic membrane to the small footplate of the stapes. The principle is the same as that used in hydraulic jacks, in which force is applied through a combination of a large and a small cylinder connected by a pipe and filled with a fluid. A small fluid pressure applied to the large cylinder results in a large pressure on the small piston. The force on the tympanic membrane is equal to the pressure times the total area of the eardrum and, assuming negligible frictional losses in the transmission through the ossicles, it is equal to the force exerted at the stapes footplate on the oval window. Because the area of the stapes is much smaller than that of the tympanic membrane, there is a *pressure amplification* given by the ratio of these areas (approximately 20:1 or 30:1).

Two other factors amplify the pressure at the stapes: a lever arm action through the ossicular chain and a buckling factor that results from the conical shape of the tympanic membrane. The potential loss in energy transmission at the air-fluid boundary is reduced considerably by the middle ear, as is evident in the 20 to 30 dB hearing loss suffered by patients in which the ossicular chain has been broken.

There are two important muscles in the middle ear: the *tensor tympani* and *stapedius*. Both muscles are attached to the ossicles. The *stapedius* muscle is innervated by motor neurons in the facial nucleus that run with cranial nerve VII, and the *tensor tympani* is innervated by motor neurons in the motor division of the trigeminal nucleus that travel in the V nerve. Contraction of these muscles increases the stiffness of the ossicular chain and can decrease the sound transmission by as much as 15 to 20 dB. Loud sounds cause the muscles to contract reflexly, a response that protects the ear from very loud sounds by reducing energy transmission.

3.3. The Inner Ear Contains the Mechanisms for Sensory Transduction

The inner ear is divided into three interconnected parts: the semicircular canals, the vestibule, and the cochlea, all of which are located in the temporal bone. Only the *cochlea* is considered here. The cochlea is the small, shell-shaped part of the bony labyrinth that contains the receptor organ of hearing. It resembles a tube that is coiled increasingly tightly on itself. One end of the tube is the apex, and the opposite end, closest to the middle ear, is the base. The fluid-filled spiral canal of the cochlea is divided along its length by a partition, the *basilar membrane*, which is attached to the bony walls of the cochlea. Cross sections of the cochlea show that the canal is divided into three ducts: scala vestibuli, scala tympani, and scala media (see Fig. 5).

The input to the cochlea is supplied by movements of the oval window by means of the stapes footplate. Because the fluid in the cochlea is incompressible, there must be some point where the pressure applied at the oval window, which is in the scala vestibuli, is transferred. This occurs at the *round window*, which is in scala tympani, through the helicotrema at the apex of the cochlea, where the scala vestibuli joins the scala tympani (Fig. 4). As the pressure wave travels the

length of the cochlea, it creates a pressure differential across the basilar membrane (between scala vestibuli and scala tympani) that sets the membrane in motion in the form of a traveling wave.

Within the scala media and attached to the basilar membrane is the receptor organ of hearing, the *organ of Corti* (Fig. 5). The organ of Corti is composed of numerous structures, most of which are not considered here. Of special importance are the receptor cells, which are similar to the hair cells in the semicircular canals (see Chapter 24). At least some of the hairs are in contact with an auxiliary structure, the *tectorial membrane*. Sound waves reaching the inner ear set the basilar membrane and the organ of Corti into motion. This motion results in a shearing force between the tectorial membrane and the hairs of the hair cells. Displacement of the hairs results in transmitter release at the base of the hair cell, where auditory nerve fibers of cranial nerve VIII make synaptic contact. All information about the acoustic environment is carried to the central nervous system (CNS) by trains of all-or-none action potentials generated in auditory nerve fibers.

The coding of acoustic information depends on the vibratory pattern of the basilar membrane. The basilar membrane is a relatively flaccid structure that

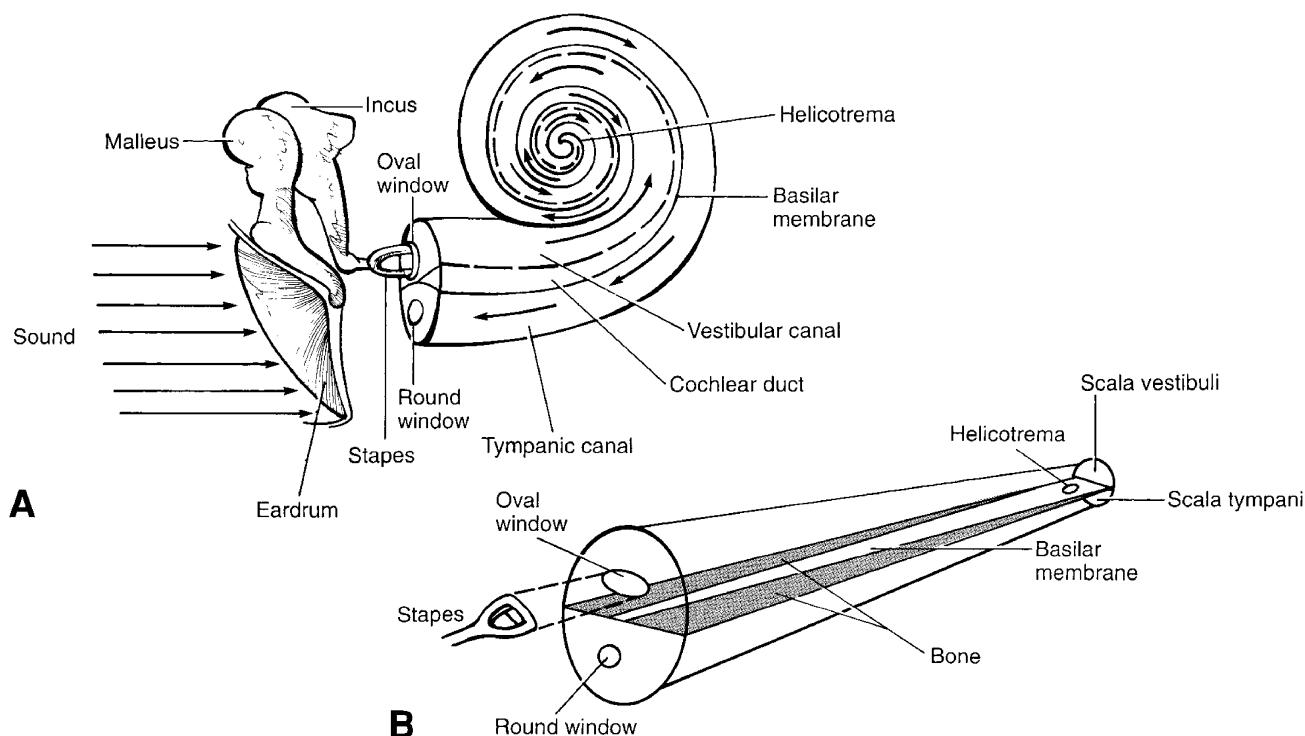


Fig. 4. The major structural features of the cochlea. **(A)** Coupling of the middle ear to the coiled cochlea through the oval and round windows. **(B)** The cochlea is shown uncoiled. The basilar membrane is narrow near the round window and wider near the helicotrema, a taper opposite to the cross-sectional area of the cochlea.

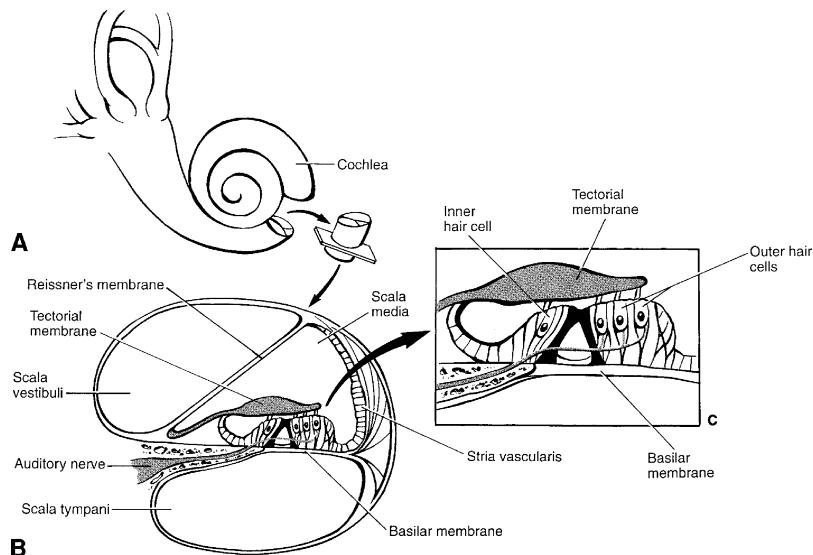


Fig. 5. Anatomic features of the cochlea. **(A)** The cochlea in relation to the vestibular channels (partially illustrated). **(B)** Cross section of the cochlea showing the organ of Corti with the cochlear ducts (scala media, scala tympani, and scala vestibuli) as well as the placement of the basilar membrane, tectorial membrane, and auditory nerve. **(C)** The structures within the scala media.

increases in width and decreases in stiffness from base to apex. Curiously, the change in the width of the basilar membrane is the opposite of the change in width of the cochlear duct as it coils from base to apex (Fig. 4). The vibratory undulations generated in the cochlea by sound waves contain all the information about the acoustic environment that must be coded into neural information. A key factor in this process is the mechanical response to sound waves of the basilar membrane and organ of Corti. Most of the pioneering work on the vibratory patterns of the basilar membrane was done by Georg von Bekesy who was recognized with the Nobel Prize in 1960.

To understand the motion of the basilar membrane and how the frequency of a sound affects this movement, we first consider the responses to a sinusoidal stimulus (i.e., tone). Each point along the basilar membrane that is set in motion vibrates at the same frequency as the acoustic stimulus. However, the amplitude of membrane vibration varies with location along the length of the basilar membrane from base to apex, depending on the frequency of the sound. A wave motion is set up along the membrane as the fluids of the inner ear are driven by motion of the stapes. This wave motion on the basilar membrane is referred to as a *traveling wave* (Fig. 6).

Because the basilar membrane becomes wider and more flaccid as the distance from the base increases, the natural frequency of vibration (i.e., resonance) of the basilar membrane decreases toward the helicotrema. Because of this variation in stiffness, low and

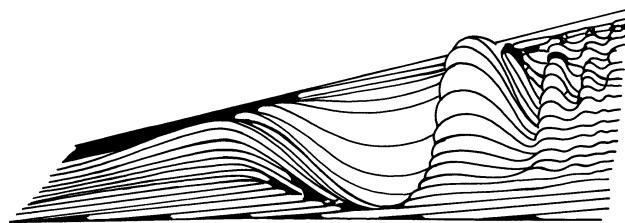


Fig. 6. Representation of a traveling wave at one instant in time. Cochlear base is at left and the apex at right. Notice the rapid decline in amplitude just to the right of the region of maximal displacement amplitude. (Redrawn from Tonndorf J. Shearing motion in scala media of cochlear models. J Acoust Soc Am 1960;32:238.)

high frequencies cause maximal vibration amplitudes along the apex and base, respectively, of the basilar membrane (Fig. 7A). If two different frequencies are received by the cochlea simultaneously, they each create a maximal displacement at different points along the basilar membrane. This separation of a complex signal into different points of maximal displacement along the basilar membrane, corresponding with the frequency of the sinusoids of which the complex signal is composed, means that the basilar membrane performs like a series of filters. The basal part of the basilar membrane responds maximally to high-frequency sounds, while the apical portion responds preferentially to low-frequency tones (Fig. 7B); the basilar membrane is tonotopically organized.

The receptor cells are hair cells that line the length of the cochlea. In most mammals, there are three rows of

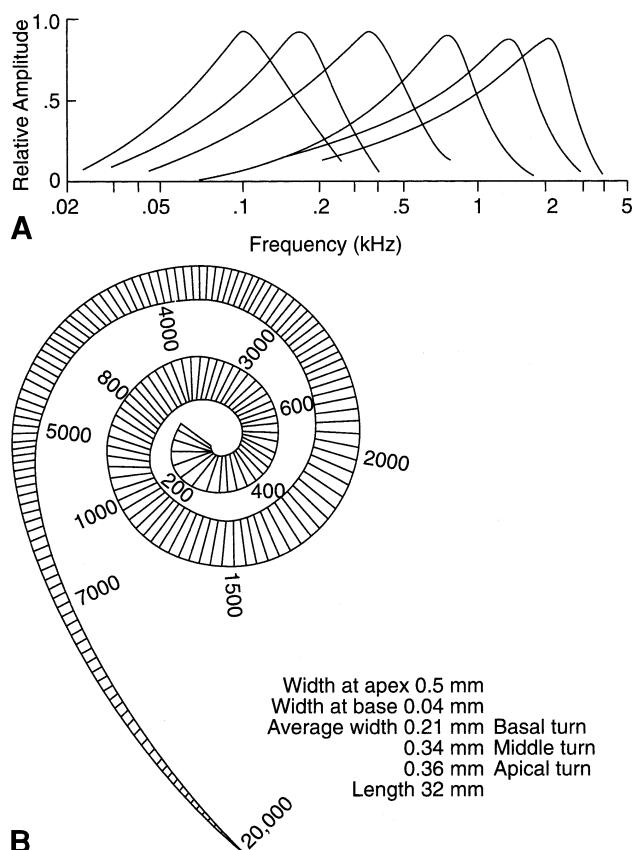


Fig. 7. (A) Envelopes of traveling waves at seven different frequencies. (Redrawn from Bekesy G von. Experiments in hearing. New York: McGraw-Hill, 1960.) (B) Diagram of the human basilar membrane, showing the approximate positions of maximal displacement to tones of different frequencies and changes in width going from the base near the stapes and oval window to the apex near the helicotrema. The ratio of width to length is exaggerated to show the variation in width more clearly. (Redrawn from Stuhlman O. An Introduction to Biophysics. New York: Wiley, 1943.)

outer hair cells and a single row of inner hair cells. The tops of the outer hair cells are embedded in the overlying tectorial membrane (Fig. 5), but it is unknown whether the same is true of the inner hair cells. When the basilar membrane is set into motion by an acoustic stimulus, the basilar membrane and tectorial membranes do not move in unison, because they pivot about different points. The result is that a shearing force is applied to the cilia of the hair cells, which bends the cilia. Just as with the hair cells in the semicircular canals, this mechanical action results in a depolarization or hyperpolarization of the membrane potential of the cell, depending on the direction of movement. The afferent auditory nerve fibers of cranial nerve VIII make chemical synapses onto the base of the hair cells. Depolarization of the hair cell increases the resting impulse discharge of the auditory

nerve fiber, while hyperpolarization from movement in the opposite direction decreases the resting discharge level.

We do not understand the exact role of the two types of hair cells. A clue can be obtained from considering their innervation by auditory nerve fibers, because the pattern is quite different for the inner and outer hair cells. In the cat, there are about 50,000 auditory nerve fibers. Not all of these are afferent fibers; about 5% are efferent fibers that have cell bodies in and around the superior olivary complex and convey information back to the cochlea. The precise nature of this efferent innervation of the cochlea is not well understood. Of the remaining afferent fibers, about 95% terminate only on inner hair cells (Fig. 8) so that each inner hair cell is contacted by about 20 afferent fibers; these are the type I afferents. The remaining type II afferent fibers innervate the outer hair cells in a much more diffuse manner, typically crossing over to the rows of outer hair cells and traveling toward the base before innervating a number of outer hair cells in all three rows. It is reasonable to conclude that the inner hair cells are most important for conveying information about the vibrations in the cochlea to the CNS.

Exactly what role the outer hair cells play and why there should be three times as many is not understood. Current speculations center on their role in efferent innervation and evidence for an active mechanism in the cochlea. Evidence for an active process comes from the surprising observation that the cochlea can emit sound spontaneously or in response to acoustic stimulation. The outer hair cells are motile and can

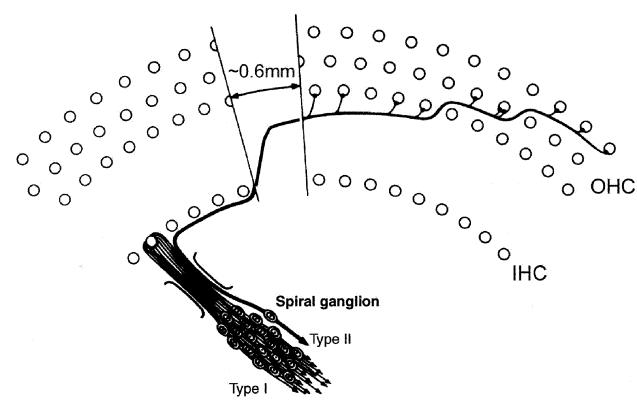


Fig. 8. Schematic view of cochlear innervation for afferent fibers in cat. IHC, inner hair cells; OHC, outer hair cells. (Redrawn from Spoendlin H. Structural basis of peripheral frequency analysis. In Plomp R, Smoorenburg GF (eds.). Frequency Analysis and Periodicity Detection in Hearing. The Netherlands: AW Sitjhoff, 1970:2.)

be made to contract when stimulated electrically. One hypothetical scenario is that the efferent system enables the CNS to contract the cilia of the outer hair cells, changing the micromechanical sensitivity of the basilar membrane to low-level stimuli. This remains an active area of research.

Because the eighth cranial nerve afferent fibers are distributed along the whole length of the basilar membrane, a tone of one frequency will excite the afferents connected to the region undergoing suprathreshold vibration. The fact that a discrete population of fibers is activated by a pure tone and that this population changes when the stimulus frequency changes is the basis for the *place principle* of hearing. This principle states that the perceived pitch of a sound depends only on the particular population of nervous elements activated. The tonotopic organization of the array of auditory nerve terminals along the basilar membrane is maintained in all major areas of the central auditory system, but this tonotopic organization is probably

not precise enough to explain a listener's ability to discriminate one frequency from another.

4. FREQUENCY TUNING AND TEMPORAL INFORMATION ARE TRANSMITTED BY AUDITORY NERVE FIBERS

There are several ways in which information about an acoustic stimulus is coded in the discharges of fibers of the auditory nerve. These results come from studies in which fine micropipette electrodes are used to record from single auditory nerve fibers.

4.1. Tuning Properties

Auditory nerve fibers are responsive only within a restricted range of frequencies and intensities. This frequency-intensity domain is called the response area, which may be considered analogous to the receptive field in the visual and somatic sensory

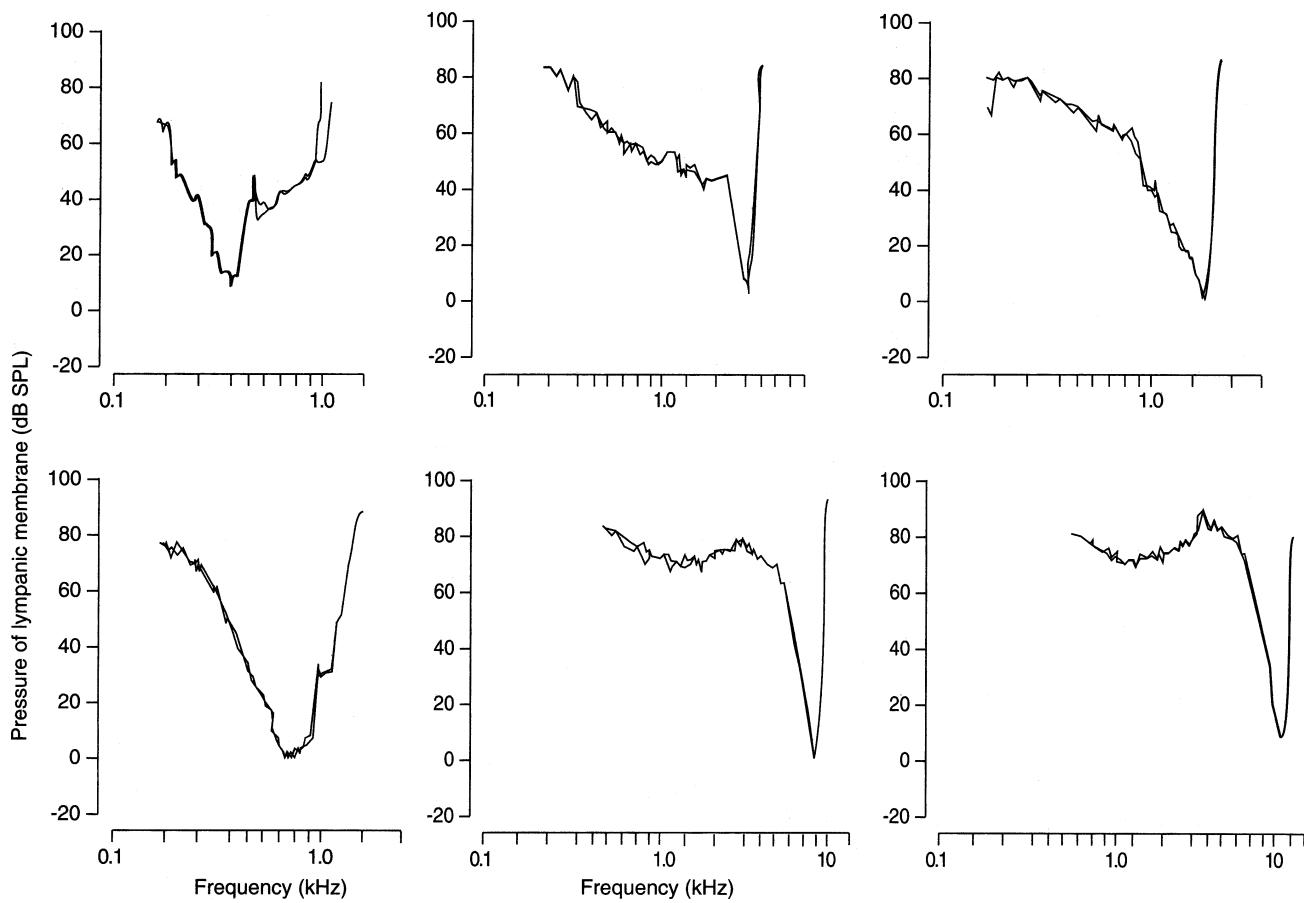


Fig. 9. Representative tuning curves (i.e., frequency threshold curves) of cat auditory nerve fibers are shown for six different regions. In each panel, two fibers from the same animal of similar characteristic frequency and threshold are shown, indicating the constancy of tuning under such circumstances. (Redrawn from Liberman MC, Kiang NYS. Acoustic trauma in cats: cochlear pathology and auditory-nerve activity. Acta Otolaryngol Suppl 1978;358:1.)

systems. A plot of the threshold sound intensity versus stimulus frequency is called the *tuning curve* of the fiber (Fig. 9). The frequency of lowest threshold is designated as the best or characteristic frequency. Different fibers have different best frequencies, reflecting their connections along the basilar membrane. Fibers with high best frequencies are connected to basal regions of the cochlea, and fibers with low best frequencies are connected more toward the apical regions.

The CNS receives information about stimulus frequency in terms of which fibers are activated in accord with the place principle of pitch perception. However, at even moderate intensity, the tuning curve is relatively broad, especially on the low-frequency side. If the place principle were the only mechanism that allowed pitch identification by the CNS, we could assume that all of the activity is ignored except at the peak or that the place principle operates only near the threshold of hearing. Because neither possibility seems reasonable, there must be other mechanisms for coding acoustic information. The *time principle* states that the acoustic waveform is encoded in the temporal discharge pattern of auditory nerve fibers.

4.2. Temporal Properties

Information about the frequency of low-frequency tones is conveyed to the CNS by another method. When the fiber discharges, it tends to do so around the same phase of the stimulus waveform (Fig. 10), corresponding with movements of the basilar membrane that move the hair bundles of the hair cells in the excitatory direction. This phenomenon is called *phase-locking* and in mammals is observed only at frequencies below about 4000 Hz. This temporal coding of low-frequency sounds is important because the energy in speech signals is predominately below 4000 Hz (Fig. 2), and the changes in sound pressure resulting from complex sounds consisting of many low-frequency spectral components are encoded in the temporal discharge in a similar fashion. With phase-locking, the time interval between spikes tends to be a multiple of the period of the stimulating tone. No single fiber fires on every cycle of a tone, but if we consider an array of many fibers, the phenomenon known as *volleying* is revealed. In the total array of responding fibers, there are discharges in some fibers on each of the stimulus cycles. Information about the period (and hence the frequency) of a low-frequency tone is represented in the temporal rhythm of nerve impulses. The CNS uses these rhythms to

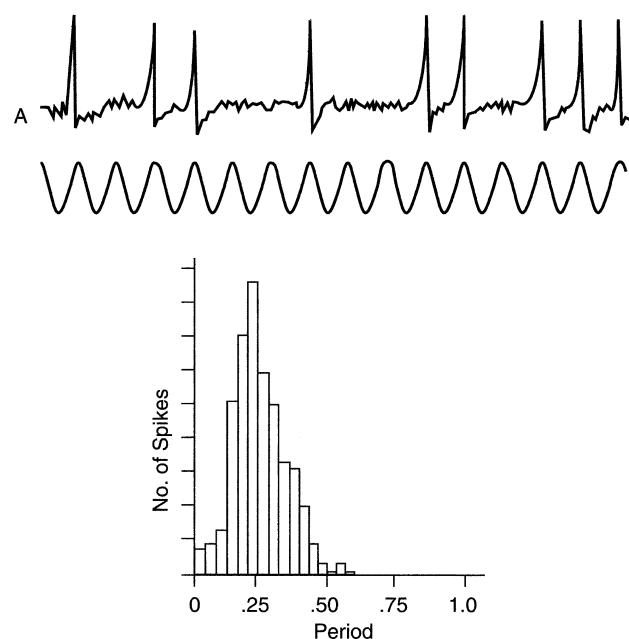


Fig. 10. Representation of the phase-locked response of a single auditory nerve fiber to a low-frequency tone. The period histogram below it shows the distribution of phase angles at which the nerve preferentially discharges.

encode speech sounds and to localize a sound source in space. Mechanisms by which the timing of neural volleys from the two ears is used in sound localization are described in the following sections.

5. CENTRAL AUDITORY SYSTEM

5.1. The Central Auditory Pathway Consists of Many Relay Nuclei

The central auditory pathway comprises a number of nuclear groups within the medulla, pons, midbrain, thalamus, and cerebral cortex. These cell groups are interconnected by fiber tracts that ascend from the cochlea to the auditory cortex (Fig. 11) and carry information about the acoustic environment that reaches consciousness. A descending pathway carries information back to the cochlea, primarily to the outer hair cells, but we do not understand the function of these descending pathways. Other pathways that communicate acoustic reflexes that are activated by sound stimulation and involve various motor systems to move the head, eyes, and ears are not described here.

5.1.1. COCHLEAR NUCLEUS

The cochlear nucleus is composed of a complex of cell groups on the lateral surfaces of the medulla

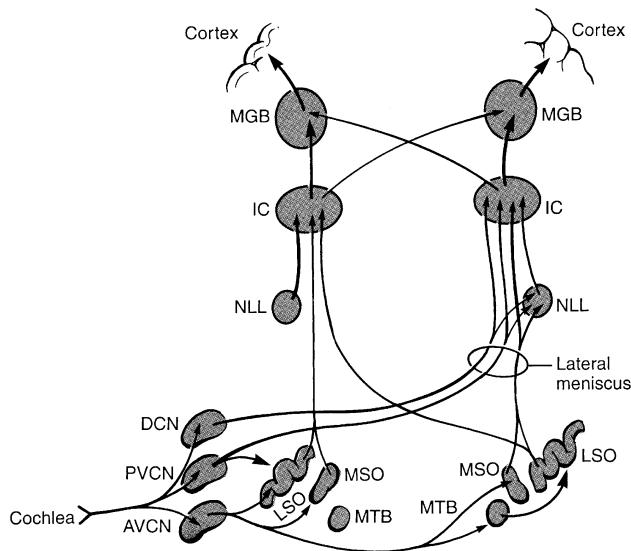


Fig. 11. The ascending (afferent) neuronal chain from the cochlea to the cortex: spiral ganglion, cochlear nucleus, superior olfactory complex, inferior colliculus, and medial geniculate. Notice the many crossed pathways that allow interactions between the outputs of the two ears. AVCN, anteroventral cochlear nucleus; DCN, dorsal cochlear nucleus; IC, inferior colliculus; LSO, lateral superior olive; MGB, medial geniculate body; MSO, medial superior olive; MTB, medial nucleus of trapezoid body; NLL, nucleus of lateral lemniscus; PVCN, posteroventral cochlear nucleus.

within which all auditory nerve fibers terminate. Entering auditory nerve fibers bifurcate in an orderly way, sending an ascending branch to innervate cells in the *anteroventral cochlear nucleus* (AVCN) and a descending branch to innervate neurons in the *posteroventral* (PVCN) and *dorsal cochlear nuclei* (DCN). The ascending auditory pathways coming out of the cochlear nucleus to the lower brain stem show a bewildering combination of bilateral and unilateral connections. For example, the AVCN projects bilaterally to the medial superior olive but only unilaterally to the ipsilateral lateral superior olive. From the medial superior olive, there is an ipsilateral projection to the inferior colliculus, but the lateral superior olive projects to the inferior colliculi of both sides. As we will see below, these particular connections do make sense functionally because they are the basis for the representation of contralateral space in the auditory system.

5.1.2. SUPERIOR OLIVARY COMPLEX

The superior olfactory complex lies in the tegmentum of the pons and consists of several subdivisions. One of the most significant aspects of the superior olfactory complex is that it is the first point at which

information from the two ears converge. Evidence shows that the different subdivisions of the superior olfactory complex are involved in different aspects of the processing of the acoustic signal, especially for binaural processing. The superior olfactory complex projects to the inferior colliculi of both sides by way of the lateral lemniscus and to the nuclei of the lateral lemniscus. The superior olfactory complex also is the source of the efferent projection back to the cochlear hair cells.

5.1.3. INFERIOR COLICULUS

The inferior colliculus comprises the caudal pair of protuberances that make up the roof of the midbrain. Although the superior colliculus is primarily visual in function, the inferior colliculus is an important auditory relay station. It receives input from the cochlear nuclear complex, superior olfactory complex, nuclei of the lateral lemniscus, and inferior colliculus of the opposite side. All fibers ascending in the *lateral lemniscus* synapse in the inferior colliculus.

Many neurons in the inferior colliculus, like those in the medial superior olive, are sensitive to extraordinarily small differences in the time of arrival of the stimulus at the two ears or small differences in interaural intensity. Like all of the other main ascending auditory nuclei, the inferior colliculus is tonotopically organized. Although the inferior colliculus sends its axons centrally only as far as the medial geniculate body, it receives an impressive descending projection from auditory cortex. The output of the inferior colliculus travels by way of the brachium of the inferior colliculus to innervate the medial geniculate body of the thalamus on the same side.

5.1.4. MEDIAL GENICULATE BODY

The medial geniculate body represents the thalamic relay for auditory information. Its neurons project in an orderly fashion to the auditory areas of the cerebral cortex by way of the sublenticular portion of the internal capsule. The auditory cortex projects back on the medial geniculate body in a highly organized way. The medial geniculate body is also tonotopically organized.

5.1.5. AUDITORY CORTEX

The cortical auditory receiving areas in humans and other primates are located on the dorsal surface of the superior temporal lobe. In humans, it occupies one or more of the transverse gyri of Heschl in Brodmann's areas 41 and 42. The auditory cortex is not uniform in its cellular architecture. It consists of a primary receiving area that is tonotopically organized

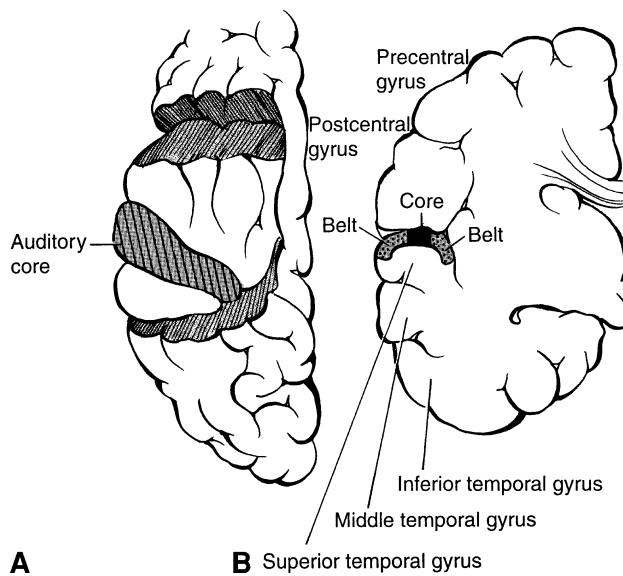


Fig. 12. The human auditory cortex in the first transverse gyrus of the temporal lobe. **(A)** The parietal lobe has been removed to reveal the superior temporal plane as seen from above. **(B)** A frontal section passes through the auditory cortex, showing the core area (A1) buried in the sylvian fissure, surrounded by a belt area of auditory association cortex. (Redrawn from Neff WD, Diamond IT, Casseday JH. Handbook of Sensory Physiology: Auditory System: Physiology (CNS): Behavioral Studies: Psychoacoustics. Berlin: Springer-Verlag, 1975:307.)

and that has a relatively uniform cellular structure throughout. Several other auditory cortical fields can be identified surrounding the auditory core (Fig. 12). Each auditory cortical area has complex afferent and efferent connections with the thalamus, with nearby cortical fields, and with auditory areas in the opposite hemisphere. The functional significance of multiple cortical areas is unknown, although it has been suggested that each processes a different aspect of the acoustic stimulus.

5.2. Central Auditory Neurons Show Some Common General Properties

A microelectrode inserted into the auditory nerve or a central auditory nucleus records trains of action potentials that are evoked by sounds reaching the ears. This method has enabled study of the way in which acoustic information is encoded in trains of nerve spikes and in the transformations that take place in these spike trains at successive levels of the auditory system. Many of the response properties of auditory nerve fibers are also observed in neurons throughout the auditory pathway. However, numerous transformations in the sound-evoked discharge

take place at each successive synaptic station as the result of convergence of excitatory and inhibitory activity from various sources.

One obvious transformation is illustrated in Fig. 11. Notice that the projection from the cochlear nucleus to the superior olivary complex is bilateral, as is the projection from the superior olivary complex to the inferior colliculus. This means that, starting from the superior olivary complex, most auditory neurons respond to stimulation of either ear. The nature of binaural interactions is discussed later.

In general, most central auditory neurons have a best or characteristic frequency. Their response areas and tuning curves share similarities with those of auditory nerve fibers, but many differences are also apparent. One general property found in many central auditory neurons is the presence of *inhibitory sidebands* in the response area. Sidebands are presumably the result of inhibitory circuits that allow cells responding to a particular frequency to inhibit the responses of other cells that are tuned to neighboring frequencies. These sidebands limit the response areas of single cells to narrower frequency ranges and are reminiscent of the center-surround receptive fields of visual neurons.

Another common characteristic of all the major auditory nuclei is a tonotopic organization. The distribution of best frequencies of single neurons can be studied systematically by microelectrode recording techniques. The resultant three-dimensional map is an orderly arrangement of best frequencies that is referred to as *tonotopic organization*. This organization is a reflection of the preservation of the orderly projection of eighth cranial nerve fibers from the cochlea to the CNS. Because frequency representation is related to the spatial distribution of points of maximal displacement along the basilar membrane, this organization also can be referred to as *cochleotopic*. It can be viewed in much the same way as retinotopic organization in the visual system and somatotopic organization in the somatic sensory system.

5.3. The Information Is Transformed in the Cochlear Nucleus

The fibers of the cochlear nerve terminate in an orderly way within the three cochlear nucleus subdivisions, the AVCN, PVCN, and DCN, preserving the innervation pattern along the basilar membrane. The result is that each subdivision is tonotopically organized.

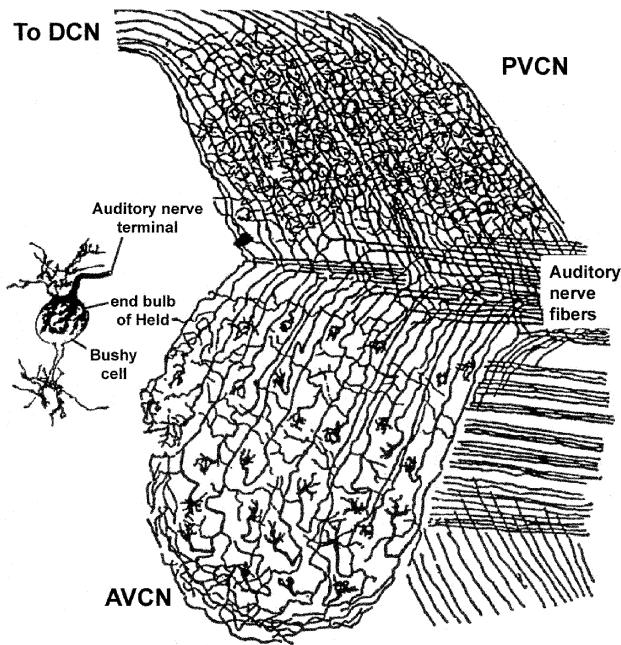


Fig. 13. Axons of the cochlear nerve entering ventral cochlear nuclei. Each axon branches to send an ascending branch to the anteroventral (AVCN) nucleus, which is characterized by specialized end bulbs and limited convergence from the cochlea, and a descending branch to the dorsal (DCN) and posteroventral (PVCN) nuclei. Notice the difference in the morphology of the terminals and the orderly, cochleotopic arrangement of fibers. (Adapted from Histologie du système nerveux. Vol. 1. Madrid: Instituto Ramon y Cajal, 1909.)

The morphology of the cells and auditory nerve terminals within each subdivision determine the kind of information that is relayed to higher auditory centers. Cells in the PVCN and DCN receive bouton endings from auditory nerve fibers and from interneurons and higher centers. This neuronal network results in interactions of excitation and inhibition that are reflected in the complex discharge patterns of single neurons, which differ considerably from the incoming volleys in eighth cranial nerve fibers. Some bushy cells in AVCN receive very specialized endings (e.g., *end bulbs of Held*) from just a few auditory nerve fibers. Figure 13 shows one of these end bulbs making contact with a “bushy cell” in the AVCN. Notice the unique morphology of the synaptic ending, which ensures that an action potential on the auditory nerve fiber is transmitted with great temporal fidelity to the AVCN. As a result, the activity of these bushy cells has been found to be similar to the activity in incoming auditory nerve fibers. In this case, the bushy cells serve as relay cells, with little transformation of the input. The end bulb synapse on bushy cells is an example of a unique specialization

in the auditory system, which is thought to preserve timing information.

An important function of the bushy cells in the AVCN is to relay the precise low-frequency time information carried by auditory nerve fibers. In fact, the convergence of a few end bulb synapses on each bushy cell has been found to actually enhance the ability of bushy cells to encode the temporal information as compared to their auditory nerve inputs. This temporal information is relayed primarily to the superior olivary complex, which is the first region in the brain where outputs from the two ears converge. On the other extreme are cells in the DCN, which only respond to acoustic inputs with a single spike or with some complex pattern of discharges that bear no simple relation to the acoustic stimulus. Some intrinsic circuitry in the DCN or the membrane properties of these cells are responsible for a different kind of transformation. We do not understand the reasons for all of these different transformations, and the only one addressed in this chapter is that of the bushy cell in the AVCN. Already at the first relay station, the auditory and visual systems manifest different processing schemes. Most of the lateral geniculate neurons cells relay the signals from the retinal ganglion cells, but only a subset of the cochlear nucleus cells can be considered to be relay cells.

5.4. Binaural Interactions Are Important for Sound Localization

In analyzing the anatomic connections of the central auditory system, it has been emphasized that some cells receive input from both ears. What are the advantages of having two ears, instead of one? If one ear is plugged, it is clear that many acoustic tasks are not affected. It is still possible to understand speech, perceive music, and discriminate sounds of different pitch. However, there are certain tasks that are much more difficult to perform with only one ear. Two tasks especially depend on binaural hearing: the ability to localize the source of sound and the ability to differentiate one sound from background noise. Psychophysical tests have demonstrated that localizing a sound source along the horizontal plane depends largely on the differences in the sound reaching the two ears. Related to this is the ability to attend to a single sound source in noisy environment, which is commonly referred to as the “cocktail party” phenomenon. The latter ability is a common complaint of patients suffering from presbycusis (i.e., progressive, bilateral, symmetric hearing loss in the elderly).

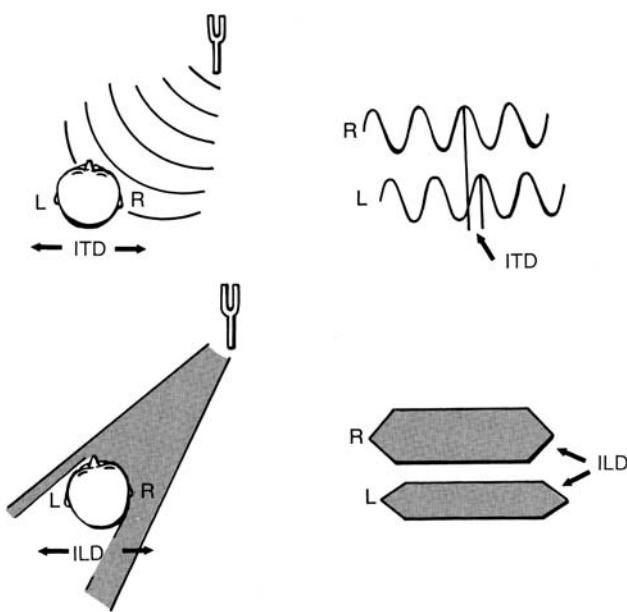


Fig. 14. The drawings illustrate interaural time differences (ΔT) for a low-frequency tone (top) and level differences (ΔL) for a high-frequency tone (bottom) when a sound source is off the midline. Those on the left are schematic diagrams of the physical situation; those on the right are the signals received at the right (R) and left (L) ears. For the high-frequency tone, only the envelope of the sine curve is shown. ITD, interaural time difference; ILD, interaural level difference.

There are two important cues for sound localization: *interaural level differences* (ILDs) and *interaural time differences* (ITDs), as shown in Fig. 14. A listener employs one or both cues in localizing a sound source. For low-frequency tones (below about 1000 Hz), a listener relies on ITD cues for sound localization, while for higher frequency tones, the listener relies on ILDs. This dichotomy between the cues used for localizing high- and low-frequency tones is known as the *duplex theory of sound localization*, which is valid only for pure tones. Corresponding with the duplex theory, the ability to localize pure tones is worst in the mid-frequency range where neither cue is very effective and improves at both higher and lower frequencies. The localization of complex sounds, which contain both high and low frequencies, uses both mechanisms. Because fundamentally different computations are necessary for encoding ITDs and ILDs, this processing would be expected to use different anatomic structures and physiologic mechanisms. This is the case in the superior olivary complex: some cells are important for encoding ITDs and others encode ILDs, and the circuitry is different for each.

Acoustically, the ears can be regarded as a pair of holes separated by a spherical obstacle, the head. Sound on one side of the head reaches the farther ear some 30 μ s later for each additional centimeter it must travel. If we assign a radius of about 20 cm to the spherical head, the maximal ITD is about 600 μ s, though the actual maximal ITD in humans is about 800 to 900 μ s. This maximal ITD occurs if the stimulus is located directly to one side, when it is as far as possible from the opposite ear.

The ability of the auditory system to detect ITDs is quite remarkable. Under optimal conditions, the just noticeable difference in ITD in humans is 6 μ s. This is accomplished in a nervous system that uses action potentials whose width and synaptic delays are more than 100 times longer! Animals specialized for acoustic communication can do even better; bats can detect arrival times of echoes of less than 1 μ s. Preserving timing information is clearly an important task for the central auditory system. How does the auditory system achieve this discrimination? The end bulbs of Held are one anatomic specialization for preserving timing information.

ILDs are not so simply behaved. The far ear lies in a sound shadow whose depth depends on the direction and wavelength of the sound. The head acts as an effective acoustic shadow only if the wavelength of the sound is smaller than the size of the head. Thus, the head can act as an effective acoustic shadow only at higher frequencies. For the size of human heads, interaural intensity differences are negligible at frequencies below about 1000 Hz and may be as great as 20 dB at 10 kHz.

For several of the auditory nuclei, spatial maps of the acoustic environment have been described. Such maps are fundamentally different from the topographic maps of the body and retinotopic maps of the visual world seen at all levels of the somatosensory and visual systems. Auditory space does not map onto the cochlea, which differentiates it from the sensory maps of somesthesia and vision that are direct projections of the sensory surface. The auditory map is said to be a *computational map*, because it must be derived from neuronal processing of the cues, the ITDs and ILDs, that produce the map.

Studies of the encoding of sound localization cues in the central auditory system have shown that, above the level of the superior olivary complex, the representation of auditory space is primarily of the contralateral sound field. Patients with lesions of the auditory cortex, for example, are unable to localize sounds originating from the contralateral sound field.

The auditory system derives a map of the contralateral field just as the visual and somatosensory maps in the CNS are of the contralateral visual field and body. The contralateral representation in the auditory system is derived from the particular way in which some fibers cross and others remain uncrossed in the ascending projection from the cochlear nucleus to the inferior colliculus. There is order behind the seemingly haphazard interconnections of the various auditory nuclei.

5.5. The Physiology of the Auditory System Is Reflected Clinically

5.5.1. BRAIN-STEM AUDITORY EVOKED RESPONSE

One of the clinically important consequences of the preservation of temporal information by the peripheral and central auditory system is that it allows a record of the *auditory brain-stem response (ABR)*. This record consists of complex, time-locked, electrical slow waves, which can be recorded from the scalps (i.e., noninvasively, like electroencephalographic patterns) of human subjects in response to a brief, abrupt sound, such as a click or tone burst (Fig. 15). These electrical potentials are extremely low in amplitude (some $<1 \mu\text{V}$) and are usually lost within the higher-amplitude spontaneous activity recorded on the electroencephalogram (EEG). However, by using computer signal-averaging techniques, it is possible to extract from the EEG a well-defined complex waveform whose peaks and troughs can be associated with activity in various parts of the auditory system.

Several components of the auditory evoked potential have been somewhat arbitrarily divided into three epochs: early-, middle-, and long-latency components. The early components, usually defined as those occurring during the first 8 to 10 ms after stimulus onset, are thought to represent activation of the cochlea and auditory nuclei of the brain stem, and it is these early components that are of interest clinically. The middle and late components represent a mixture of activity in the brain stem and higher auditory centers and in the association cortex.

During the past few years, the early components have been employed in evaluating hearing loss and in helping to diagnose trauma or disease involving the brain stem. This technique is proving to be important in assessing the hearing of persons unwilling (e.g., malingeringers) or unable (e.g., newborns, mentally retarded) to cooperate in routine audiometric testing. The ability to identify the activity of specific auditory nuclei by the presence of characteristic waves of the ABR is of great clinical value. Many of the children

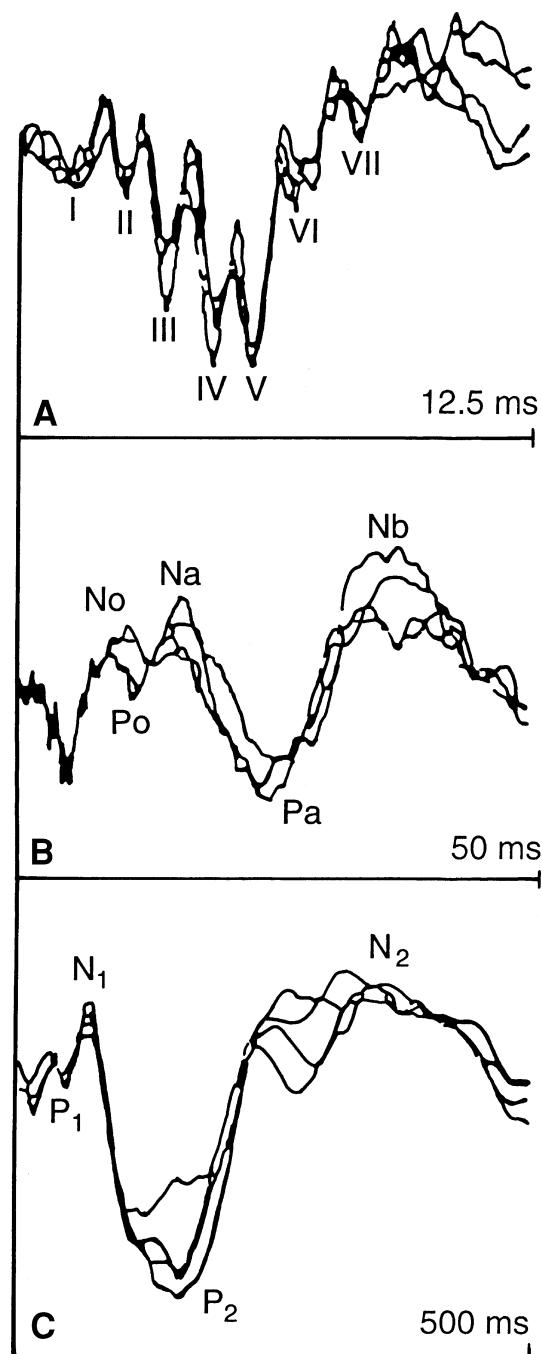


Fig. 15. Scalp-evoked potentials shown on three different timescales. **(A)** Auditory brain-stem responses (ABRs). **(B)** Middle components. **(C)** Late components. Electrodes are vertex to mastoid (positive up). Each trace shows the average of 1024 clicks (60 dB SL) delivered at 1/s to the right ear. Labels shown are commonly used for individual wave components. (Redrawn from Picton TW, Hillyard SA, Krausz HI, Galambos R. Human auditory evoked potentials. *Electroenceph Clin Neurophysiol* 1974;36:179.)

who had previously been thought to be “slow learners” or developmentally disabled were instead found to be suffering from hearing loss. The absence or degradation of acoustic input during the first few years, when the recognition and expression of speech is so critical, can cause permanent developmental damage, which can be prevented by restoring the acoustic experience.

The reason that such analysis can be done in the auditory system and not in, for example, the visual system is that the specialization within the auditory system to preserve timing information of the stimulus results in a synchronous activation of many cells at each of the early brain-stem nuclei. This synchrony is able to be detected by computer averaging even when recording from the scalp of a subject.

5.5.2. CONDUCTIVE AND SENSORINEURAL DEAFNESS

Anything that interferes with the transmission of sound to the hearing apparatus or of the neural signal to the auditory cortex can produce deafness. There are two major categories of deafness: conductive and sensorineural deafness. *Conduction deafness* is caused by a malfunction in the transmission of sound from the outer to the inner ear. Usually this happens with problems in the middle ear. A common example, particularly in young children, is the buildup of fluid in the middle ear because of infection or otitis media. Treatment with antibiotics has greatly reduced the incidence of conductive hearing loss from otitis media. In adults, otosclerosis is the most frequent cause of conduction deafness, in which there is overgrowth of the labyrinthine bone around the oval window, leading to fixation of the stapes. This condition can often be treated by surgical intervention to loosen or replace the stapes. Boosting the input to the ear with a hearing aid alleviates many cases of conduction deafness.

Sensorineural or nerve deafness is caused by damage to the cochlea, to the auditory nerve, or to the central auditory system. There are many possible causes of sensorineural deafness. The hair cells of the inner ear are especially susceptible to damage from prolonged exposure to loud sounds, antibiotics (e.g., streptomycin, kanamycin, gentamicin), or rubella infection *in utero*. The most common type of hearing loss in the aged, presbycusis, is characterized by a progressive loss of high frequencies, which is probably caused by progressive changes in the cochlea. In the central auditory system, any damage to the pathways leading from the cochlear nucleus to the auditory cortex can result in deafness. Acoustic neuroma, a tumor that develops in the auditory nerve

in the internal auditory meatus or at the cerebellopontine angle, produces an ipsilateral deafness, tinnitus (i.e., sensation of ringing in the ears), or vestibular problems. Not much can be done about most cases of sensorineural deafness.

Because many cases of sensorineural deafness involve problems in the cochlea without involvement of the auditory nerve fibers or central connections, it may be possible to stimulate the auditory nerve fibers electrically. Much attention has been focused in recent years on the development of a cochlear prosthesis in which an electrode is implanted directly into the cochlea. Electrical stimulation of the *cochlear implant* can activate the still viable ends of auditory nerve fibers, and some sense of hearing can be restored.

It is important to differentiate conductive from sensorineural deafness, because many types of conductive deafness can be treated. This is particularly important in young children during the critical time during which they are learning to speak. Two simple tests with tuning forks are helpful. They both rely on the fact that the middle ear can be bypassed when the sound is delivered directly to the bone. Air conduction usually is much more efficient than bone conduction, but in cases of conductive deafness, bone conduction can become more efficient. *Weber's test* can be used when a patient complains of deafness in one ear. A vibrating tuning fork (usually 512 Hz) is applied to the forehead at the midline, and the resulting vibrations are conducted through the bone to each ear. Patients with conduction deafness report that the sound is heard louder in the deaf ear, presumably because bone conduction is equal in the two ears but the deaf ear also hears no background noise from the air. Patients with sensorineural deafness cannot hear the sound even through bone conduction and report that it is louder in the normal ear. In *Rinne's test*, the tuning fork is applied to the mastoid process. As soon as the sound ceases to be heard via bone conduction, the tip of the tuning fork is moved to just outside the auditory meatus. In a normal ear, the fork is heard again when the sound waves travel by air conduction, because it is more efficient than bone conduction. In conduction deafness, the fork's vibration remains imperceptible when held next to the ear.

5.5.3. EFFECTS OF LESIONS IN AUDITORY CORTEX

Lesions of the auditory cortex make up a special case of sensorineural deafness. Many behavioral experiments have been conducted using cats, and extensive damage to these areas has little effect on the animal's ability to discriminate between tones of different frequencies or intensities. The major effect

appears to be on the animal's ability to localize the source of a sound in space and to discriminate between complex sound patterns.

There have been numerous reports in the literature on the effects of temporal lobe damage in humans. Unfortunately, in only a few cases have complete audiometric tests been made and postmortem examination of the brain carried out. It is not surprising that reports have conflicted on the effects of temporal lobe damage on hearing in humans. However, several effects usually are detected in humans after auditory cortical damage. After a period following bilateral lesions of the auditory cortex, the pure tone hearing threshold appears to return toward normal, and there is usually little or no change in threshold. There is also

little or no change in discrimination of alterations in intensity or frequency. Deficiencies are detected more commonly for the discrimination of changes in the temporal order or sequence of sounds, their duration, or in the ability to localize sounds in space.

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A. Tucker Gleason

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1. INTRODUCTION

The vestibular system developed in order to facilitate an organism's movement through its environment. The functional organization of this system is a remarkable network of reflexes and behaviors that allow for the stabilization of visual cues during movement, the establishment of posture during resting states (e.g., sitting or standing), and the maintenance of body balance while in motion. This arrangement necessitates interactions among vestibular structures of the inner ear (the *semicircular canals* and *otolith organs*), the ocular motor system, the visual system, the motor control system, as well as proprioceptive input regarding position and movement of the body and limbs. The interplay of these systems allows the individual to accomplish actions ranging from those as seemingly simple as standing still to those as intricately controlled as figure skating or gymnastics, and everything in-between.

Disturbances of vestibular function may lead to vertigo, ataxia, and falling. These are among the most common symptoms encountered in clinical medicine, with nearly 40% of all people in the United States experiencing at least one of these symptoms by age 65 years. Although a vestibular disorder can

occur at any age, a majority of people in the United States over age 70 years report symptoms of dizziness, imbalance, or falling. Fall-related injuries and the quality-of-life impact of vestibular disorders are the bitter concerns of these patients, as well as their family members. Dizziness and balance disorders constitute significant public health issues, particularly as people are living longer and enjoying good health well into their eighties. The careful assessment of vestibular function is imperative for effective management of the balance-disordered patient.

2. VESTIBULAR REFLEXES

The two major signals for what is known as “the sense of balance” that originate in the inner ear are related to *head movement* and *head position*. The interpretation of these signals by the central nervous system gives rise to reflexes for the control of compensatory eye movements, posture, and balance. In its most basic form, the organization of the vestibular system can be considered in two general categories of reflexes: *vestibulo-ocular* and *vestibulospinal*.

2.1. *Vestibulo-ocular Reflexes*

Vestibulo-ocular reflexes developed to stabilize the visual environment during movement of the head, thereby minimizing loss of visual acuity, and to integrate visual motion cues necessary for competent

balance. Vestibulo-ocular reflexes occur in response to angular and linear movements of the head, as well as to changes in the orientation of the head with respect to gravity. These reflexes involve sensory structures of the peripheral vestibular system, as well as central connections and pathways of the brain stem, medial longitudinal fasciculus, and nuclei that contain the motor neurons innervating extraocular muscles, with substantial direct and indirect influence of the cerebellum. The most notable of these vestibular-mediated eye movements is known as the *vestibulo-ocular reflex* (VOR). Simply stated, the VOR is a compensatory eye movement that is an exact replication of a head movement, but in the opposite direction. In other words, when the head rotates to the right, the eyes will deviate to the left; when the head rotates upward, the eyes will deviate downward, and so forth. The VOR is initiated by stimulation of the semicircular canals.

2.2. Vestibulospinal Reflexes

Vestibulospinal reflexes are necessary for achieving static posture and maintaining balance during movement. These reflexes involve interactions with motor control systems of the brain-stem reticular formation, red nucleus, subthalamic nucleus, substantia nigra, and basal ganglia, as well as cortical influences via the pyramidal system. The effector organs of vestibulospinal reflexes are the so-called antigravity extensor muscles of the neck, trunk, and extremities. The vestibulospinal reflexes function to counteract changes in the center of gravity during movement or translation. Vestibulospinal reflex activation is initiated by stimulation of the otolith organs, with small contributions of the semicircular canals.

The vestibulospinal pathways include the medial vestibulospinal tract, with fibers originating primarily in the medial vestibular nucleus; the lateral vestibulospinal tract, with fibers originating primarily in the lateral vestibular nucleus; and the reticulospinal tract, with fibers originating in the brain-stem reticular formations.

The most notable of the several spinal reflexes related to vestibular function is the *vestibulospinal reflex*, or VSR, in which acceleration of the head results in specific stereotypical limb movements, and as such, makes the VSR a functional equivalent of the VOR. Activation of the VSR causes extension of limbs ipsilateral to the direction of head acceleration and contraction of limbs contralateral to the direction of acceleration. Other reflexes with vestibular contributions are the *righting*, the *myotatic*, and

the *functional stretch* reflexes. The righting reflex functions to maintain the head in a horizontal orientation with respect to gravity, independent of trunk movement. During linear translations, the head will move in a direction opposite to that of the trunk in order to maintain horizontal head position. The myotatic reflex functions to maintain stability or stiffness at a joint. In this reflex, stimulation of muscle proprioceptors increases the activity of muscle spindle afferents, which in turn recruit α -motor neurons resulting in muscle contraction. As with the myotatic reflex, the functional stretch reflex is triggered by muscle proprioceptors and functions to coordinate limb and trunk movements across joints. In the context of the vestibular system, sway or perturbation (particularly in the anterior-posterior dimension) will produce functional stretch reflexes about the ankle, knee, or hip in order to maintain postural stability. If the perturbation exceeds the limits of sway imposed by the body's center of gravity over the feet, the reflex will be used to generate a step response in order to avoid a fall.

3. THE VESTIBULAR RECEPTORS

The vestibular receptors of the semicircular canals and otolith organs respond to head acceleration.

The structures of the membranous labyrinth that are associated with vestibulo-ocular and vestibulospinal reflexes are the three pairs of *semicircular canals* (the horizontal [or lateral], anterior [or superior], and posterior) and the *otolith organs* (the *utricle* and *saccule*) (Fig. 1). As in the cochlea, these structures are filled with endolymph.

3.1. Semicircular Canals

The semicircular canals are oriented nearly at right angles to one another, resulting in the representation of the three rotational dimensions of space. When the head is tilted approximately 25° forward, the horizontal canal is in the horizontal plane, and the anterior and posterior canals, also known as the vertical canals, are in vertical planes. Functionally, the coplanar semicircular canal pairs consist of the right anterior canal and left posterior canal, the left anterior canal and right posterior canal, with the right and left horizontal canals forming the third functional pair.

The widened end of each of the semicircular canals is known as the *crista ampullaris*, or *ampulla*. The neurosensory epithelium of each ampulla is composed of supporting cells and two types of hair cells. Projecting from each hair cell are *stereocilia* (40 to 110

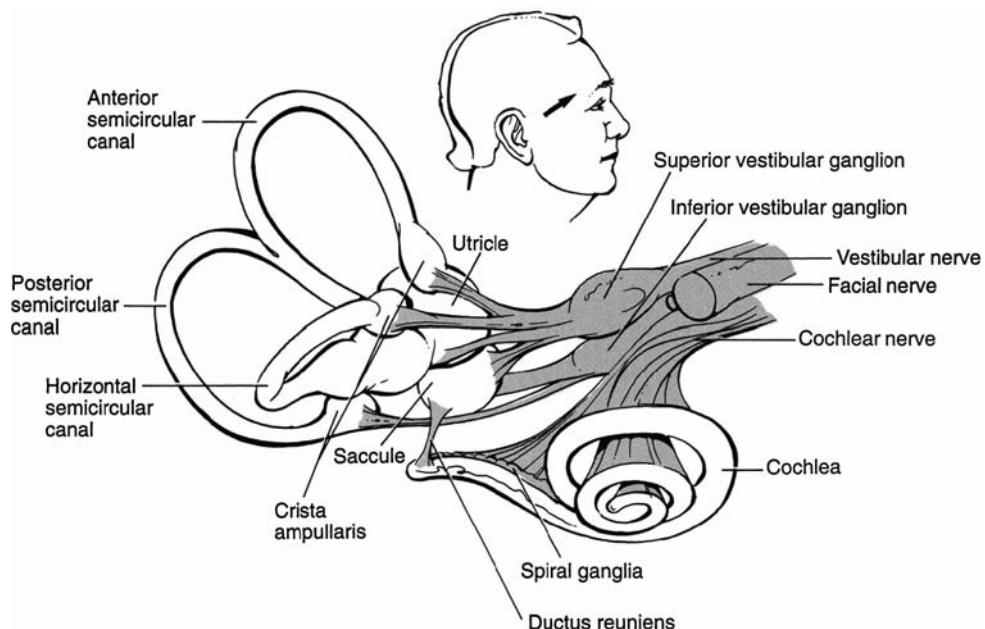


Fig. 1. Components of the membranous labyrinth: the three semicircular canals (i.e., horizontal, anterior, and posterior), the otolith organs (i.e., saccule and utricle), and the cochlea. The spiral-shaped cochlea winds around a central bony modiolus. Bipolar neurons in the spiral ganglion innervate hair cells in the organ of Corti, and their central processes form the cochlear nerve. Peripheral processes of bipolar neurons in the superior and inferior vestibular ganglia innervate hair cells in the ampullae of the semicircular canals and the maculae of the saccule and utricle, and their central processes form the vestibular nerve. The arrow indicates the plane of the horizontal semicircular canal.

per cell) and a single *kinocilium* (Fig. 2). These cilia are embedded in a gelatinous structure called the *cupula* that extends to the roof of the ampulla. The stereocilia for each hair cell have graded lengths that increase in the direction of the kinocilium. As in the cochlea, vestibular hair cells are activated or inhibited by deflection of the cilia. Specifically, each vestibular hair cell is activated when its cilia are displaced toward the kinocilium and is inhibited with displacement in the opposite direction. All hair cells in the ampulla of a single semicircular canal are arranged in the same fashion, with all kinocilia of that ampulla positioned on the same side of the hair cell. This imparts a morphologic axis of polarization for each semicircular canal, by which deflection of the cilia either toward or away from the kinocilia ultimately results in a corresponding increase or decrease, respectively, in the neural firing rate for that canal. In the ampullae, the cilia are deflected by movement of the cupula caused by the flow of endolymph through the canals resulting from head movement, more specifically, by *angular acceleration* of the head. The activation plane of each semicircular canal comprising a functional pair is a mirror image of its counterpart, by which head movement in that plane will result in an increased neural firing rate in one canal and a decreased firing rate in the other.

3.2. Otolith Organs

The *utricule* and *saccule* have major roles in facilitating *vestibulospinal reflexes*, with smaller contributions serving *vestibulo-ocular reflexes*. The *utricle* is oriented almost horizontally, and the *saccule* is oriented almost vertically in the sagittal plane, resulting in the representation of linear acceleration vectors produced by gravity or by translation of the head. The *utricle* and the *saccule* each contain a *macula* (Fig. 3). The neurosensory epithelium of each macula consists of supporting cells and hair cells, which have bundles of stereocilia and a single kinocilium. These hair cells are embedded in the *otolithic membrane*, which is a gelatinous matrix containing *statoconia* (crystalline calcium carbonate), making the otolith organs responsive to inertial and gravitational forces. In the maculae of the *utricle* and *saccule*, the cilia are deflected by movement of the otolithic membrane resulting from an appropriate *linear acceleration* of the head or head tilt. The eye movements mediated by the otolith organs are those related to linear components of head acceleration, which serve to stabilize visual images with changes in viewing distance, and ocular counter-roll, which occurs in response to sustained head tilt. The contributions of otolith organ stimulation to vestibulospinal reflexes will be discussed later.

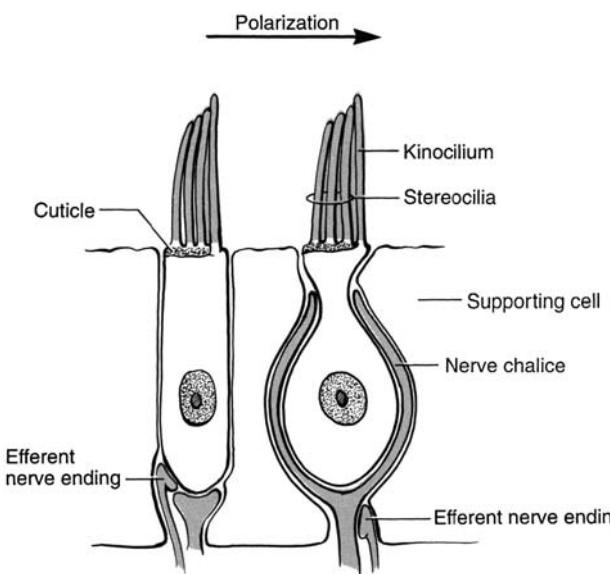


Fig. 2. Two principal types of hair cells in the ampullae of the semicircular canals and the maculae of the otolith organs. Type I hair cells are goblet-shaped and are innervated by chalice-type nerve endings that arise from large-diameter axons of bipolar neurons in the vestibular ganglion. Type II hair cells are columnar and have simple nerve endings associated with small-diameter axons at the base of the cells. Vestibular efferent nerve endings establish synaptic connections with the primary afferent ending on type I hair cells and directly on the base of type II hair cells. Both types of hair cells have an array of stereocilia that are polarized according to increasing length in the direction of the kinocilium. In the ampullae, the stereocilia and kinocilia are embedded in a gelatinous cupula, and in the maculae, they contact the otolithic membrane with its crystalline statoconia matrix. The structural and innervation differences between the two types of hair cells are correlated with differences in their physiologic activity.

The macula of the utricle is located on the floor of the utricle. When the head is upright, the utricular macula is oriented almost parallel with the ground. The posterior end is horizontal, whereas the anterior end is elevated about 45° from horizontal. As in the ampullae of the semicircular canals, the hair cells in the utricular macula are arranged with morphologic axes of polarization, but in a more complex fashion (Fig. 3). Half of the macula is stimulated by displacement of the otolithic membrane in one direction, but the other half is inhibited, and the opposite is true for the opposite direction. The landmark known as the *striola* corresponds with the maximal thickness of the overlying layer of statoconia and marks the reference point of the polarization axes of the hair cells. The utricular macula is stimulated predominately by linear forces near the horizontal plane, such as are encountered while accelerating or decelerating in a car.

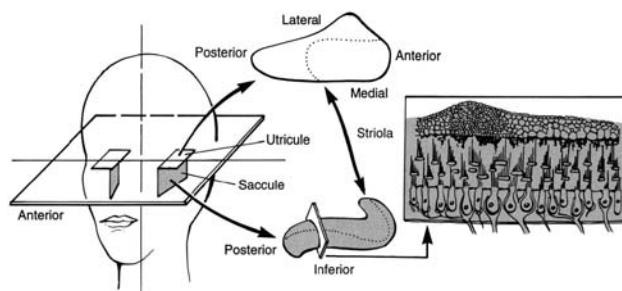


Fig. 3. Spatial orientation of the utricle and saccule. The maculae of the utricle and saccule are curved, plate-like structures on which the hair cells are polarized in opposite directions in relation to the striola, which corresponds with the maximal thickness of the overlying layer of statoconia. In the macula of the utricle, which is oriented approximately parallel to the ground, the hair cells are polarized *toward* the striola on each side. In the macula of the saccule, which is oriented approximately perpendicular to the ground, the hair cells are polarized *away from* the striola. The orientation and shape of the maculae of the utricle and saccule and the polarizations of their respective hair cells effectively provide a means of responding to linear accelerations in three dimensions.

The macula of the saccule is an elongated structure that lies perpendicular to the ground when the head is upright. Hair cells in the saccular macula also have a complex arrangement within the neurosensory epithelium (Fig. 3). The vertical orientation of the saccular macula suggests that the cilia are deflected optimally by vertically directed linear forces of the head, such as are encountered while riding in an elevator.

A closer look at vestibular hair cells reveals that they are morphologically similar to inner and outer hair cells in the organ of Corti within the cochlea. *Type I hair cells* of the vestibular system are spherical or goblet-shaped (similar to inner hair cells in the cochlea) and are innervated by a large chalice-like afferent ending that encompasses most of the cell (Fig. 2). These cells have large-diameter afferent axons and typically respond to strong stimulation. *Type II hair cells* are smaller and cylindrical (similar to outer hair cells in the cochlea), and afferent and efferent endings are distributed at the base of the cell. These cells are innervated by small-diameter afferent axons and appear to respond to weak stimulation.

The innervation of hair cells in the semicircular canals and the otolith organs is provided by *bipolar neurons* in the vestibular (Scarpa's) ganglion, which lies at the base of the internal auditory meatus. Peripheral processes from neurons in the superior division of the ganglion provide innervation for the anterior and horizontal semicircular canals and the maculae of the utricle and part of the saccule. Bipolar neurons in the inferior division of the ganglion innervate hair cells

in the posterior semicircular canal and most of the macula of the saccule. The central processes of these bipolar neurons form the superior and inferior branches of the vestibular portion of the vestibulochlear, or eighth, cranial nerve. The information transmitted by vestibular nerve axons is encoded in the frequency of discharge of primary vestibular axons. Topographic organization, like that in other sensory systems (e.g., somatotopic, retinotopic, tonotopic), is not apparent in the vestibular system.

4. CENTRAL VESTIBULAR CONNECTIONS AND PATHWAYS ARE RELATED TO THE CONTROL OF EYE MOVEMENTS, BALANCE, AND POSTURE

The vestibular nerve courses through the cerebellopontine angle medial to the cochlear nerve and enters the brain stem between the inferior cerebellar

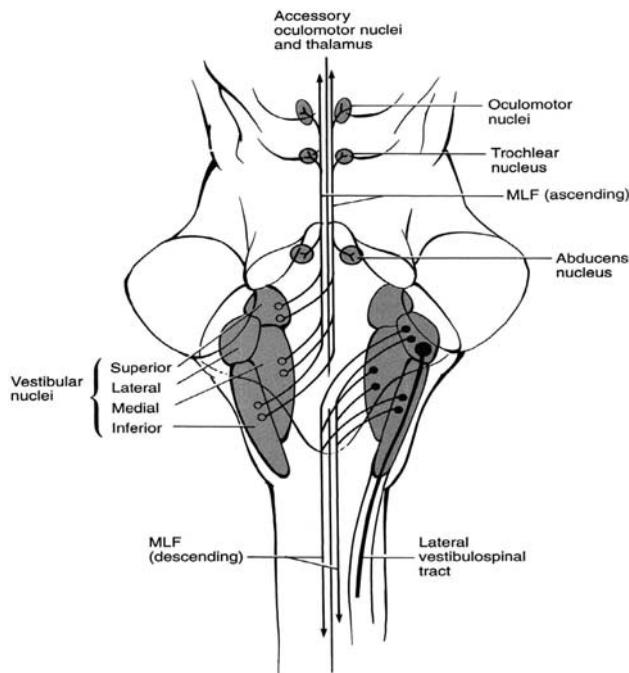


Fig. 4. The ascending (left) and descending (right) vestibular pathways. Ipsilateral inhibitory and contralateral excitatory ascending projections from the vestibular nuclei course through the medial longitudinal fasciculus (MLF) and target motor neurons in the abducens, trochlear, and oculomotor nuclei. The ascending connections mediate vestibulo-ocular reflexes. Bilateral descending fibers in the MLF project primarily to motor neurons in the cervical spinal cord that innervate dorsal neck muscles, forming the basis for the vestibulo-collic reflex. The lateral vestibulospinal tract is formed from the lateral brain-stem vestibular nuclei and descends ipsilaterally in the spinal cord, primarily targeting motor neurons that innervate axial extensor muscles that maintain posture.

peduncle and the spinal trigeminal tract. Most vestibular fibers terminate in one or more of the brain-stem vestibular nuclei (Fig. 4). These nuclei lie in the floor of the fourth ventricle and extend from a level rostral to the hypoglossal nucleus to slightly beyond the level of the abducens nucleus. The nuclei of the brain-stem vestibular complex are arranged in two longitudinal columns. The lateral column consists of the inferior (i.e., spinal or descending) vestibular nucleus, the lateral vestibular nucleus of Deiters, and the superior vestibular nucleus of Bechterew. The medial (i.e., triangular) vestibular nucleus of Schwalbe constitutes the medial cell column.

On entering the brain-stem vestibular complex, most primary vestibular fibers bifurcate into ascending and descending branches. In general, primary vestibular fibers that innervate the ampullae of the semicircular canals project predominately to rostral portions of the brain-stem vestibular complex, including the superior vestibular nucleus, and portions of the lateral and medial vestibular nuclei. The primary vestibular fibers that innervate the maculae

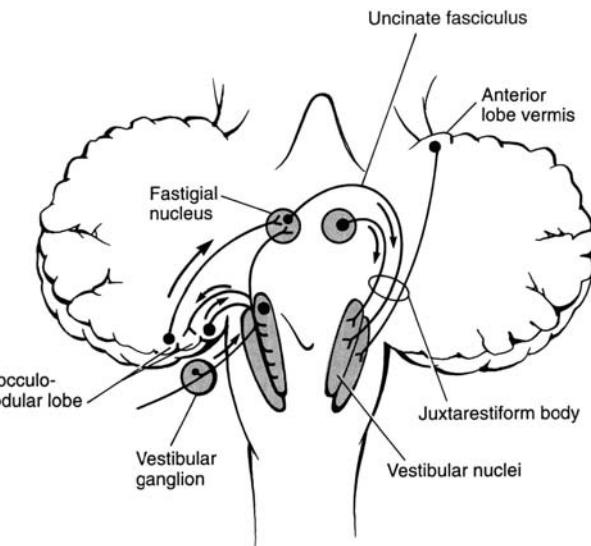


Fig. 5. Vestibulocerebellar and cerebellovestibular connections. First-order fibers from bipolar neurons in the vestibular ganglion and second-order fibers from neurons in the brain-stem vestibular nuclei project to the flocculus, nodulus, and uvula of the cerebellar cortex (i.e., vestibulocerebellum) and to the fastigial nucleus by way of the juxarestiform body. Purkinje cells in the cerebellar cortex project directly and indirectly by the fastigial nucleus to the brain-stem vestibular nuclei through the juxarestiform body. Purkinje cells in the vermis of the anterior lobe of the cerebellum (i.e., spinocerebellum) project directly through the juxarestiform body to neurons in the lateral brain-stem vestibular nucleus that are the cells of origin of the lateral vestibulospinal tract.

of the otolith organs terminate in caudal portions of the medial and inferior brain-stem vestibular nuclei. Some primary vestibular fibers bypass the brain-stem vestibular nuclei and ascend to the cerebellum through the *juxtaprestiform body* (Fig. 5). Cells in all parts of the vestibular ganglion send projections to the ipsilateral *nodulus* and *uvula* of the cerebellar vermis for integration with projections from the visual system to coordinate combined eye-head movements under visual and vestibular influences.

4.1. Second-Order Vestibular Connections

Unlike the auditory system and most other sensory systems, the central connections of the vestibular system are related primarily to *motor* behaviors involving the maintenance of balance and compensatory movements in response to changes in head and body position. These functions are achieved by connections of the brain-stem vestibular nuclei with motor neurons

in the extraocular motor nuclei, the spinal cord, and the cerebellum.

4.1.1. MEDIAL LONGITUDINAL FASCICULUS

The superior, medial, and inferior brain-stem vestibular nuclei are the main sources of fibers that compose the medial longitudinal fasciculus (MLF). Ascending fibers in the MLF from these nuclei project primarily to the oculomotor, trochlear, and abducens nuclei, which contain the motor neurons innervating the extraocular muscles, as well as to accessory oculomotor nuclei (Fig. 4).

4.1.2. ASCENDING MLF

Second-order vestibular fibers in the MLF are concerned with two general functions: compensatory adjustments of the eyes relative to the head and neck; and maintained postural deviation of the eyes, under the control of the utricle and saccule, during linear acceleration to produce compensatory conjugate

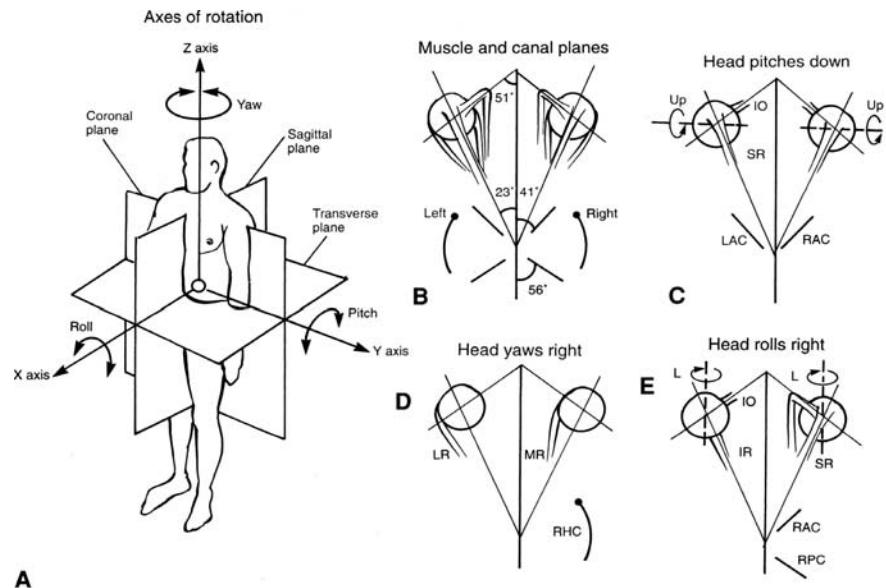


Fig. 6. Compensatory eye movements produced by movements of the head in the vestibulo-ocular reflex. **(A)** The planes of reference and axes of rotation. **(B)** Angular relationship between the orientation of the semicircular canals and the pulling actions of the vertical extraocular muscles. The orientation of the anterior semicircular canal approximates the pulling action of the superior rectus muscle, and the orientation of the posterior semicircular canal approximates the pulling action of the superior oblique muscle. The orientation of the horizontal semicircular canal approximates the pulling actions of the lateral rectus and medial rectus muscles. **(C,D,E)** Activation of different pairs of extra-ocular muscles resulting from rotation in different axes. **(C)** When the head is pitched down, the anterior semicircular canals (LAC, RAC) are excited bilaterally, producing a compensatory upward eye movement that results from activation of inferior oblique (IO) and superior rectus (SR) motor neurons. **(D)** A head turn to the right excites the right horizontal semicircular canal (RHC) and inhibits the left horizontal semicircular canal (LHC), which produces a compensatory eye movement to the left resulting from activation of the left lateral rectus (LR) and right medial rectus (MR) muscles. **(E)** A roll movement to the right coactivates the anterior and posterior semicircular canals (RAC, RPC) on the right side, producing a compensatory torsional eye movement achieved by activating the inferior oblique (IO) and inferior rectus (IR) muscles in the left eye and the superior oblique (SO) and superior rectus (SR) muscles in the right eye.

vertical and torsional eye movements. The compensatory eye movements are made in response to the combination of stimulation of the semicircular canals brought about by head rotation (i.e., angular acceleration) and proprioceptive impulses arising in the dorsal neck muscles that course through the spinovestibular tract. The proprioceptive input terminates in the inferior vestibular nucleus from which information is relayed to the motor nuclei of the extraocular muscles via the MLF.

The ascending fibers of the MLF serve vestibular-mediated eye movements. Second-order vestibular fibers that cross the midline and ascend contralateral are excitatory, and those that are uncrossed and ascend ipsilateral are inhibitory. The connections of second-order vestibular neurons with motor neurons in the extraocular motor nuclei are specific to the semicircular canal of origin and are related to the spatial orientation of the canals and their alignment with the pulling actions of the corresponding extraocular muscles (Fig. 6B). Excitatory second-order vestibular neurons that receive input from the posterior semicircular canal project to inferior rectus motor neurons in the oculomotor nucleus and superior oblique motor neurons in the trochlear nucleus, controlling vertical, downward eye movement. Excitatory second-order vestibular neurons that receive input from the anterior semicircular canal project to superior rectus and inferior oblique motor neurons in the oculomotor nucleus, controlling vertical, upward eye movement (Fig. 6C). Excitatory second-order vestibular neurons that receive input from the horizontal semicircular canal project directly to lateral rectus motor neurons in the abducens nucleus and indirectly to medial rectus motor neurons in the oculomotor nucleus by a relay neuron in the abducens nucleus, thereby controlling horizontal eye movements (Fig. 6D). Torsional eye movements, such as occur in response to roll movements, are produced by coactivation of the anterior and posterior semicircular canals on the same side (Fig. 6E). In almost all cases, the motor neurons that innervate the antagonistic muscles are inhibited in the same canal-specific manner. These vestibulo-ocular reflexes function to stabilize visual images on the retina despite movement of the observer, the object of visual regard, or both.

4.1.3. DESCENDING MLF

Descending fibers in the MLF originate from the medial and inferior brain-stem vestibular nuclei and establish synaptic connections predominately with motor neurons in the cervical spinal cord that innervate muscles of the neck and control movements of

the head (Fig. 4). The descending limb of the MLF also is called the *medial vestibulospinal tract*, particularly with reference to fibers that project caudal to cervical levels. Some second-order vestibular neurons have axons that bifurcate and project to the extraocular motor nuclei and the cervical spinal cord. These connections form the basis of the *vestibulo-collic reflex*, in which activation of dorsal neck muscles occurs in response to rotation of the head or rotation of the body when the head is held stationary. The purpose of this reflex is to stabilize the position of the head during movement.

4.1.4. LATERAL VESTIBULOSPINAL TRACT

The lateral vestibulospinal tract arises from neurons that are located in the lateral vestibular nucleus (Fig. 4). Only the ventral portion of the lateral vestibular nucleus receives direct primary vestibular input from the semicircular canals. The dorsal region of the nucleus has connections with the vermis of the anterior lobe of the cerebellum, which is the site of termination of the dorsal spinocerebellar tract. The projection from the anterior cerebellar vermis to the lateral vestibular nucleus is somatotopically organized such that cervical regions are represented rostral and lumbar regions are represented caudal in the nucleus. The lateral vestibulospinal tract descends uncrossed in the anterior funiculus of the spinal cord and establishes *excitatory* synaptic connections with motor neurons in lamina IX, primarily at cervical and lumbar levels of the spinal cord. The lateral vestibulospinal tract is involved in the regulation of posture by influencing motor neurons that innervate primarily *axial extensor muscles*.

4.1.5. RETICULOSPINAL TRACT

Only a very few primary vestibular fibers target the pontomedullary reticular formation of the brain stem. The majority of vestibular connections with the reticular formation are indirect, by way of second-order projections from all four brain-stem vestibular nuclei. Fibers of the reticulospinal tract terminate in laminae VII and VIII of the spinal cord at all levels. Reticular formation modulation and integration of vestibular signals with projections from the accessory optic tract, cerebellar efferents, and ascending spinal tracts facilitate the establishment of posture and maintenance of balance during movement.

4.1.6. VESTIBULOCEREBELLAR FIBERS

The cerebellum has a major role in the integration of vestibular and visual information. In addition to first-order vestibular ganglion neurons, some

neurons in the superior, medial, and inferior brain-stem vestibular nuclei project their axons through the juxtarestiform body bilaterally to the nodulus, uvula, and flocculus of the cerebellar cortex and to the *fastigial nucleus* (Fig. 5). Reciprocal connections with the cerebellum are achieved by cerebellovestibular fibers from the fastigial nuclei of both sides and from Purkinje cells in the ipsilateral flocculus that course through the juxtarestiform body to terminate in all four brain-stem vestibular nuclei. In this manner, the cerebellum exerts considerable influence throughout the vestibular complex, which may play an important role in recovery after loss of vestibular function from illness or injury.

4.1.7. VESTIBULAR COMMISSURAL CONNECTIONS

The vestibular nuclei on both sides of the brain stem are connected homotopically to the same nuclei on the contralateral side by direct and indirect excitatory and inhibitory commissural pathways. The direct commissural pathway courses through the dorsal tegmentum of the brain stem. Indirect connections use the vestibulocerebellar pathways and the *prepositus hypoglossi nucleus*, which is intimately related to the vestibular nuclei by extensive reciprocal connections. Vestibular commissural connections play a significant role in the phenomenon of *vestibular compensation*, which is a recovery of function from the deficits that are associated with peripheral lesions of the vestibular end organs or nerve.

4.1.8. VESTIBULAR EFFERENT CONNECTIONS

Cholinergic neurons in the vicinity of the abducens nucleus project their axons into the vestibular nerve and establish efferent connections with the hair cells in the ampullae of the semicircular canals and the maculae of the otolith organs. Synaptic connections are made indirectly on type I hair cells via the primary afferent nerve endings and directly with the cell bodies of type II hair cells (Fig. 2). Little is known about the function of the vestibular efferent pathway. Because the synaptic connections of vestibular efferent neurons with vestibular hair cells are reminiscent of those established by efferent olivocochlear neurons with hair cells in the cochlea, it seems likely that the vestibular efferent pathway is capable of modulating the activity of the hair cells during movements of the head.

4.1.9. VESTIBULAR-THALAMIC-CORTICAL PATHWAY

Some second-order fibers that project to the oculomotor and trochlear nuclei and the accessory oculomotor nuclei terminate in the *ventral posterior*

inferior nucleus of the thalamus, which is located in the vicinity of the ventral posterior somatosensory relay nuclei. The ventral posterior inferior nucleus projects to a distinct region of the postcentral gyrus, coextensive with Brodmann's area 3a. This region is thought to be responsible for complex position and movement perception, possibly by integrating vestibular information with proprioceptive information from joint afferents and group I muscle afferents.

5. NYSTAGMUS

Nystagmus is a rhythmic, oscillatory, involuntary movement of the eyes in any or all fields of gaze. It consists of two phases: a slow drift of the eyes in one direction, followed by a fast movement in the opposite direction to bring the eyes back to midline. Nystagmus is named for the direction of the fast phase, such that a slow rightward drift of the eyes followed by a fast leftward movement would be called "left-beating" nystagmus. Nystagmus is always pathologic if it occurs in the absence of stimulation or is prolonged after stimulation. It may be induced in normal persons by two different methods: rotation or caloric stimulation of the external auditory canals.

Rotational nystagmus occurs in response to angular acceleration. At the onset of rotation, the initial inertia of the endolymph in the semicircular canals in the plane of rotation deflects the cupula in one direction (Fig. 7A), resulting in the production of the VOR with the eyes moving in the opposite direction as rotation. Once the eyes reach an end point in the orbit, the fast phase will occur bringing the eyes back to midline. As nystagmus is named for the direction of the fast phase, *per-rotational* nystagmus will be in the *same* direction as the rotation. If the rotation continues, the endolymph eventually attains the same velocity of rotation as the semicircular canal. When this occurs, the cupula will return to its resting position, therefore, stimulation ceases and nystagmus is no longer produced (Fig. 7B). If the rotation suddenly stops, the inertia of the endolymph continues its flow, with a renewed stimulation of the vestibular cupula, but in the opposite direction (Fig. 7C), such that *post-rotational* nystagmus will be in the *opposite* direction as the rotation.

Caloric nystagmus depends on the production of convection currents in the endolymph of the semicircular canals created by a thermal gradient of the temporal bone in response to irrigating the external auditory canal with either cool or warm stimulus (typically water or air). Because of proximity of the

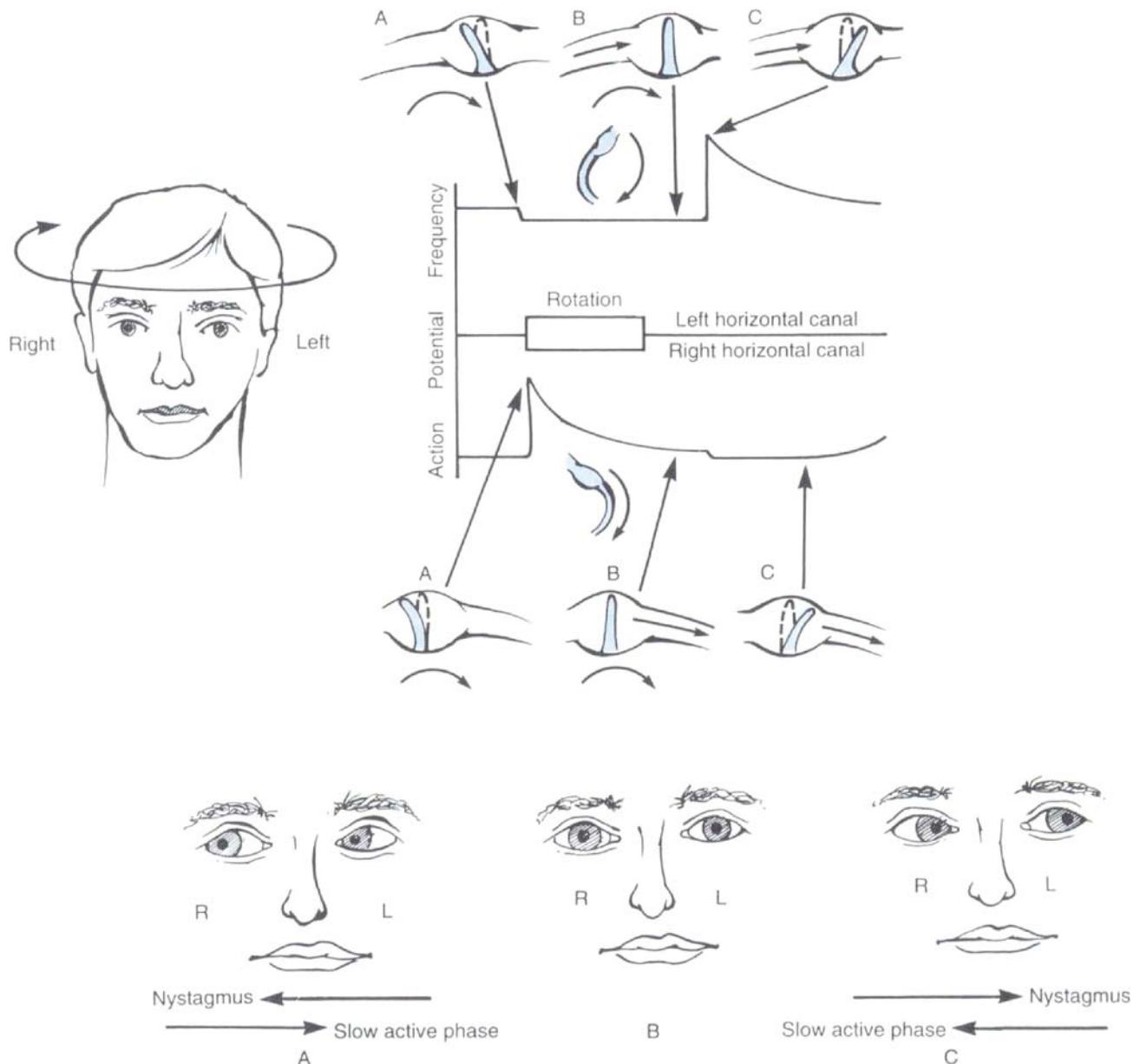


Fig. 7. Principles of rotational nystagmus at the start and during rotation, and post-rotational nystagmus after cessation of rotation. Rightward rotation of the head and body is depicted. **(A)** At the start of rotation, as the head and horizontal semicircular canals begin to move, the endolymph remains stationary and effectively causes excitation of the right horizontal semicircular canal, inhibition of the left horizontal semicircular canal, and nystagmus to the right (i.e., in the direction of rotation). **(B)** During rotation, the head, semicircular canals, and endolymph are moving at the same velocity, and the eyes remain stationary. **(C)** Immediately after stopping rotation, the head and the semicircular canals are stationary, but the endolymph continues to flow in the direction of rotation to the right, resulting in excitation of the left lateral semicircular canal, inhibition of the right lateral semicircular canal, and nystagmus to the left (i.e., in the opposite direction of rotation).

horizontal semicircular canal to the external auditory canal, it tends to be the most responsive to caloric stimulation. The head is positioned such that the horizontal canal is in a vertical position (the subject lying supine with the head elevated 30°), where convection currents created in the endolymph as a result

of caloric stimulation will have maximal influence. Irrigating the ear canal with a cool stimulus (30°C) lowers the temperature of the prominence of the horizontal canal, condensing the endolymph and inducing a circulating motion of the endolymph. This results in inhibition of the hair cells in the ampulla

of the horizontal semicircular canal, causing the eyes to deviate slowly toward the side of the irrigated ear (i.e., slow phase of the nystagmus), and then return quickly in the opposite direction (i.e., fast phase of the nystagmus). The nystagmus produced by a cool stimulus will be in the opposite direction of the irrigated ear (left-beating nystagmus with irrigation of the right ear, and right-beating nystagmus with irrigation of the left ear). If the ear is irrigated with a warm stimulus (40°C), the endolymph expands, and the resulting circulation of endolymph produces the reverse; the nystagmus slow phase is directed away from the irrigated ear, and the fast phase is directed toward the irrigated side. Hence the mnemonic COWS (cold-opposite, warm-same) for the expected direction of caloric nystagmus.

6. VESTIBULAR ASSESSMENT

The principles of rotational and caloric nystagmus are used in the assessment of individuals presenting with dizziness and balance disorders. The tests of vestibular function seen most often in clinical practice include rotary chair testing and electronystagmography (ENG) or the more recently developed technology of videonystagmography (VNG). These tests provide information regarding the origin (i.e., peripheral vs. central) and extent (i.e., bilateral or unilateral) of vestibular deficit by making use of vestibular pathways related to the control of eye movements. Dynamic posturography examines the relative contributions of vestibular, visual, and proprioceptive input in the control and maintenance of balance and posture for evaluation of vestibulospinal reflex competence.

Rotary chair testing consists of eye movement recordings made either electrographically, via the cornea-retinal potential, or videographically during and after rotational stimulation. The rotational stimulation can be either sinusoidal (i.e., to and fro) or sustained in one direction, resulting in the production of the VOR and nystagmus, which are used to evaluate overall vestibular responsiveness and symmetry.

The ENG or VNG battery is composed of tests of ocular motility, positional maneuvers of the head and body, and caloric stimulation. Recordings of the eyes are made while visual targets are presented stimulating saccadic, tracking, eccentric gaze, and optokinetic eye movements. Deficits of these eye movements generally suggest central abnormalities. The positional

subtests assess the effects of gravitational forces acting on vestibular sensors in sitting, supine, right lateral, left lateral, and head hanging positions. The presence of nystagmus in positional tests can be indicative of either central or peripheral abnormality, depending on specific characteristics of its occurrence. The slow phase of nystagmus resulting from caloric irrigation of the external auditory canals is assessed for symmetry and direction in order to distinguish unilateral or bilateral weakness of peripheral vestibular function.

In dynamic posturography, the center of mass of the person being evaluated is derived from reaction force measurements made by a force plate incorporated into the surface upon which the person stands. The person attempts to maintain a quiet stance as visual and proprioceptive cues that contribute to balance and posture are systematically manipulated in order to determine the sensory integration of vestibular, visual, and proprioceptive input. These cues may also be suddenly and unexpectedly perturbed in order to assess movement coordination of vestibulospinal reflexes during challenges of balance maintenance.

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Steven J. St. John and John D. Boughter Jr.

CONTENTS

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1. INTRODUCTION

1.1. Taste Versus Flavor

The term *taste* has a number of different connotations. Most commonly, the word is used to describe the sensations arising from chewing and swallowing food. We ask how the meal tastes, or we complain that the milk doesn't taste right. We season our food "to taste." And we may regret the loss of taste when we have the common cold.

Neuroscientists use a more restricted definition of the term. Taste (gustation) is the sensations that arise from the stimulation of the taste receptors whose neural message is conveyed by particular afferent nerves to particular central processing areas. Properly speaking, then, our enjoyment of food is a mixture of primarily taste, olfaction, and somatosensation but may also include auditory and visual components. Collectively, this complex of sensations is known as flavor perception.

1.2. The Sensations of Taste

Classically, taste has been considered to be represented by four discrete basic tastes: sweet, salty, sour, and bitter (although there has always been debate as to whether these sensations are continuous, as in the sensations of color vision). There is a growing consensus in the existence of additional primary taste qualities, however. Many psychophysicists add *umami* (savory) to the list of basic tastes, and recently,

there is evidence that fats (known to be sensed by the trigeminal and olfactory systems) may be detected by the gustatory system. Despite this relatively small list of taste qualities, hundreds of molecules are detected by taste buds.

Sweet taste is evoked by a variety of carbohydrates, most notably mono- and disaccharides such as glucose, sucrose, maltose, and lactose. Sweet taste can also be evoked by a number of other molecules, which has been exploited in the development of low-calorie or noncaloric artificial sweeteners (e.g., saccharin, aspartame, and acesulfame K). In general, sweet taste signals the presence of calories, which presumably explains why sweet tastes are strongly preferred by virtually all terrestrial animals. Although it is common to speak of "sweet taste" as a unitary experience, there is evidence that some molecules (such as sucrose vs. maltose, or sucrose vs. saccharin) evoke qualitatively different sweet tastes.

Salty taste is evoked by a variety of salts, but most purely by salts containing sodium. Sodium is a required micronutrient that is vital in maintaining the tonicity of the blood, the osmotic balance of cells throughout the body, and the electrical activity of nerve and muscle cells. Many animals prefer mild concentrations of sodium salts to high or low concentrations, suggesting that the gustatory system participates in the physiologic maintenance of salt balance. Indeed, many organisms (including humans in certain pathologic states such as adrenal gland dysfunction) will show a robust and specific salt appetite when blood levels of sodium become dangerously low.

Sour taste is evoked by organic and inorganic acids. Although many humans enjoy mildly sour foods, sour substances are avoided in proportion to their pH. Sour taste serves to evoke physiologic buffering responses (evoking salivation and altering saliva's buffering potential) and behavioral avoidance.

Bitter taste is evoked by an enormous variety of chemicals that include proteins, amino acids, non-sodium salts, and alkaloids (a very partial list). Although bitter substances are sometimes preferred by some humans at low concentrations, bitter substances evoke behavioral avoidance (disgust) in proportion to their concentration, and most may be detected at far lower concentrations than those of substances evoking the other taste qualities (e.g., at micromolar concentrations). Bitter taste often warns of the presence of toxins but is also evoked by a number of medicinal drugs. Resistance to the bitter taste of oral pharmaceuticals (particularly among children) is often a major impediment to treatment compliance (e.g., many oral antibiotics are powerfully aversive).

Umami, or savory taste, is evoked by L-type amino acids, most notably by monosodium glutamate. While there continues to be some debate as to whether this is a distinct taste quality or a mixture of, for example, sweet and salty, the discovery of taste receptors activated by monosodium glutamate has contributed to the acceptance of this "fifth" taste quality. Evolutionarily, it may help to signal the presence of protein in a foodstuff.

1.3. The Functions of Taste

Taste is naturally allied with the olfactory and trigeminal systems in regulating ingestive behavior: Sweet tastes are increasingly preferred as concentration increases, and bitter and sour substances are increasingly avoided. One of the functions of taste is undoubtedly perceptual; taste allows us to identify foods and discriminate them from one another, and thus guide appropriate ingestive responses to safe, nutritious foods and to avoid dangerous foods.

In addition to this identification/discrimination function allied with our other orosensory systems, taste may also be viewed as the oral component of a visceral afferent system, which includes gustatory, respiratory, cardiovascular, and gastrointestinal functions. Taste elicits physiologic reflexes (such as salivation and preabsorptive insulin release) and oromotor reflexes (such as chewing and swallowing, or gagging and tongue protrusion). Avidity for sweet compounds is regulated in part by phase of metabolism; avidity for salts is regulated in part by

renal hormones. Neural circuitry of the taste and viscerosensory systems run in parallel throughout the nervous system, and both are closely allied with regulatory circuitry of the autonomic nervous system.

Taste processing also involves ventral forebrain structures associated with emotion, such as the amygdala and the nucleus accumbens. Indeed, sweet tastes elicit dopaminergic activity in central regions associated with drugs of abuse. Neuroscientists thus may make technical distinctions between a taste's quality (sweet, salty, sour, bitter, umami), its hedonic evaluation (pleasant/unpleasant), and its effectiveness in evoking reflexes of a physiologic (e.g., salivation) or behavioral (e.g., orofacial gaping) nature.

1.4. Disorders of Taste

One of the surprising features of the gustatory system is that it is relatively robust to damage or disease. In part this is due to redundancy (taste information is carried to the brain by four branches of three cranial nerves) and location (taste circuits run parallel with cardiovascular and respiratory circuits, whose damage can be life-threatening). "Loss of taste" is a fairly common complaint, but in the vast majority of cases, "taste" is used in the colloquial sense, as any food-evoked sensation. More commonly, taste complaints are actually caused by diminished olfaction. Upper respiratory infections, for example, result in reduced airflow to the olfactory receptors and thereby decreased food appreciation. In fact, the perseverance of the gustatory system in the context of a diminished olfactory sense is what usually inspires "taste" complaints.

Ageusia (complete taste loss) is virtually nonexistent, but hypogeusia (decreased intensity of taste) and dysgeusia (bad or abnormal taste sensations) do occur. Conditions that cause salivary disturbances (xerostomia, Sjögren's syndrome, head/neck radiation treatment, or the use of anticholinergic medication) frequently produce hypogeusia. Oral dryness may prevent molecules from adequately reaching the taste receptors and may also impair the health of the taste buds themselves. Decreased salivary function may also underlie mild taste loss with aging. Hypogeusia or dysgeusia can result from injury to the cranial nerves or to the taste buds themselves (e.g., during dental surgery or as a result of Bell's palsy). Dysgeusias such as metallic taste are a side effect of certain drug treatments, such as those to treat hypertension.

2. PERIPHERAL TASTE SYSTEM

2.1. Taste Buds

Transduction of tastants is a function of taste receptor cells, elongated neuroepithelial cells that are packed together in spherical structures known as taste buds (Fig. 1). Taste receptor cells are polar: their

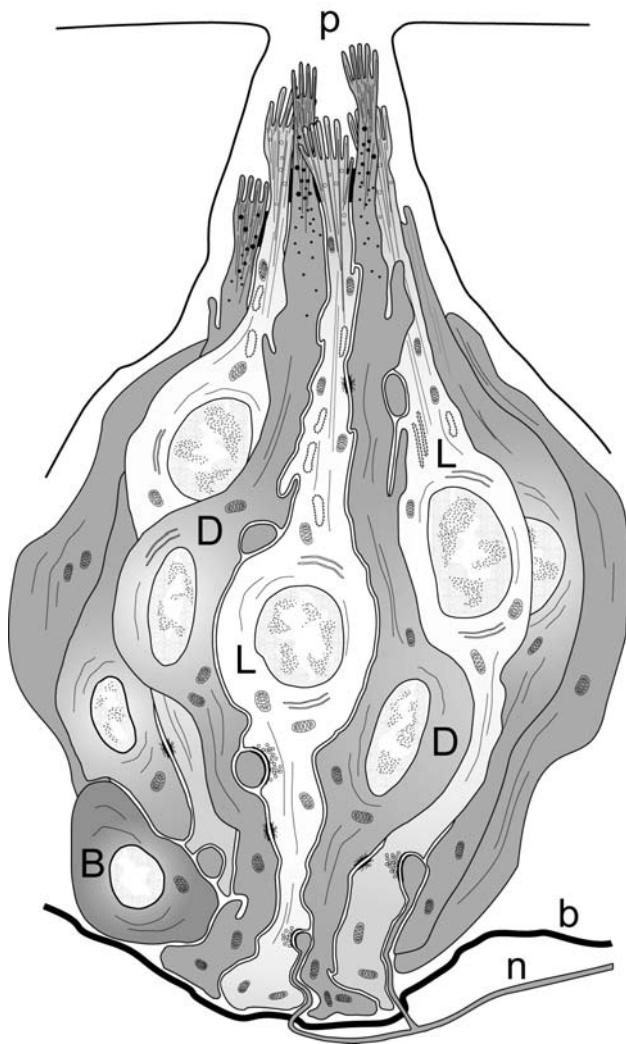


Fig. 1. Mammalian taste bud. This barrel-shaped structure contains different cell types, including basal cells (B), dark cells (D; also called type I cells), and light cells (L; also classified as either type II or type III cells). These epithelial receptor cells make synaptic contact with distal processes (n) of cranial nerves VII, IX, or X, which penetrate the basement membrane (b) to enter the basal portion of the taste bud. Cell bodies of the peripheral nerve fibers lie within the cranial nerve ganglia. The microvilli of the taste cells project into an opening in the epithelium, the taste pore (p), where they make contact with gustatory stimuli. The apical processes of the taste cells are joined by tight junctions, which restrict most taste stimuli to the apical membrane.

apical (or mucosal) domain communicates with the oral cavity through a small opening known as the taste pore, and their basal domain communicates with other cells within the taste bud or directly with afferent fibers of cranial nerves VII, IX, or X. Taste buds contain 50 to 100 cells, and it has long been known that the cells within the taste bud show anatomic differences. Small spherical cells (basal cells) that lie at the lower margin of the taste bud may serve as receptor cell progenitors. Elongate cells function as taste receptor cells; these cells may be described as dark cells and light cells based on their relative densities in electron micrographs and on several other ultrastructural characteristics. Only recently have functional differences begun to be clearly delineated among these cells, which are now classified as type I, II, and III cells based on expression profiles. It now appears that taste buds may not passively transmit information to the brain but in fact begin to process information via cell to cell communication within the bud itself.

A recent and surprising finding is that whereas taste receptors for amino acids, sugars, and bitter-tasting compounds are found on type II cells, these cells do not contain classic synaptic proteins. In contrast, type III cells, which form classic synapses with primary afferents, do not express G protein-coupled taste receptors. A model is emerging whereby a subset of cells respond to tastants and influence type III cells through nonsynaptic mechanisms. Type III cells may therefore integrate input from several other cells within the bud, finally releasing a neurotransmitter (likely adenosine triphosphate, possibly in addition to other transmitters) onto the primary afferents.

Taste buds are found on the anterior portion of the tongue in fungiform papillae and in circumvallate and foliate papillae on the posterior tongue. There are also taste buds on the soft palate, pharynx, epiglottis, and upper third of the esophagus. The distribution of taste buds on the human tongue and within the oral cavity is shown schematically in Fig. 2. In humans, the 200 to 300 fungiform papillae on the anterior portion of the tongue contain approximately 1600 taste buds, although there is considerable variation among persons. The 8 to 12 circumvallate papillae contain about 230 taste buds each, for a total of almost 3000 taste buds, and the foliate papillae have about 1300 taste buds. Although taste buds have been described on the soft palate of human adults only in biopsy material and only in very small numbers, a few studies have reported that human infants have about 2600 taste buds in the pharynx and larynx and on the

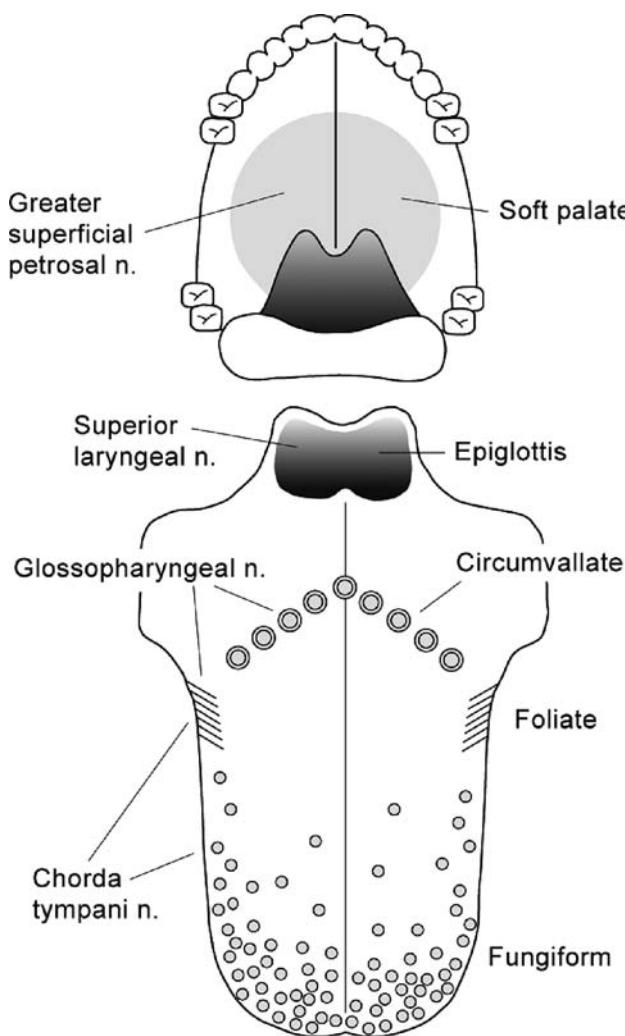


Fig. 2. Diagram of the human oral cavity, showing the distribution of various taste bud populations, which are found on the anterior tongue (i.e., fungiform papillae), posterior tongue (i.e., circumvallate and foliate papillae), the soft palate, and the laryngeal surface of the epiglottis. These taste buds are innervated by several branches of cranial nerves VII (facial nerve: chorda tympani and greater superficial petrosal branches), IX (glossopharyngeal nerve: lingual-tonsillar branch), and X (vagus nerve: superior laryngeal branch).

soft palate. On the tongue of adult rhesus monkeys (e.g., fungiform, circumvallate, and foliate papillae), there are approximately 8000 to 10,000 taste buds, which are maintained well into old age.

Few studies have determined the number of taste buds in humans, but there does seem to be a great range of individual differences. These individual differences relate to taste perception. Based first on people's perception of the bitter chemical phenylthiourea (PTC), and then on perception of a wide variety of compounds, psychophysicists have identified people

as weak tasters, medium tasters, and supertasters. These categories are distinguished by anatomic differences as well; supertasters appear to have the greatest density of taste buds (at least on the anterior tongue), and some evidence exists that supertasters have poorer eating habits (being overly driven to eat sweet-tasting foods and overly sensitive to bitter foods including vegetables).

2.2. Development, Turnover, and Regeneration of Taste Buds

In humans, the gustatory system is largely developed at the time of birth. In rats and mice, the sensory ganglia begin to form around embryonic days 8 to 10 (the gestational period of both animals is 21 days). Axons begin to emerge from the ganglia prior to the full development of the tongue and penetrate the lingual epithelium by embryonic day 14. (Axons penetrate the nucleus of the solitary tract centrally around or slightly prior to this event.) Although early studies suggested that the gustatory papillae and taste buds may be induced by sensory axons, recent work has demonstrated that both papillae and taste buds form in the absence of innervation. However, innervation is required for the maintenance of these structures; both papillae and taste buds irreversibly degenerate in the prolonged absence of innervation during the early developmental period. The gustatory system of both rodents, humans, and many other mammals is functional *in utero*; there is even evidence that food preferences can be affected by the mother's diet in several species (including humans). Appropriate orofacial reflexes (lapping to sweet tastes and gagging to bitter tastes) are present in humans at birth.

As with many systems exposed to the environment, peripheral gustatory structures undergo replacement throughout life. Taste receptor cells arise continually from an underlying population of basal epithelial cells. Estimates of the life span of a taste receptor cell vary; classic reports suggest that taste cells may turn over every 10 days. Neural innervation appears to be required for the proliferation of new taste receptor cells, but experiments in which the glossopharyngeal nerve and the chorda tympani nerve were transected and cross-anastomosed in adult rodents suggest that the differentiation of new receptor cells is guided by the local epithelium rather than the innervating nerve. The death of taste receptor cells appears to be tightly regulated by classic cell death pathways leading to apoptosis. This regulation of cell death is in contrast with the case in the surrounding epithelium.

As noted, interruption of the nerve supply in adult organisms causes the loss of taste buds. After regeneration of the peripheral nerve, however, taste buds reappear. Thus, although innervation does not appear to induce taste buds during development, reinnervation of the nerve in adults results in the return of nearly the full complement of taste buds. Experiments in rats indicate that the regenerated taste system is normal virtually immediately. The few studies of nerve repair in humans also suggest that the regenerated taste system is functional.

2.3. Taste Transduction

Taste receptor cells express a number of G protein-coupled receptors (GPCRs) and ion channels on their apical membranes. The interaction between taste stimulus and taste receptors or channels typically

produces a depolarization of the receptor cell, either directly or indirectly via second-messenger cascades (Fig. 3). The receptor potential may lead to the release of neurotransmitters, but in some taste cells it also leads to the activation of voltage-gated Na^+ and K^+ channels on the basolateral membrane, which underlie the generation of action potentials (only a small percentage of taste cells generate action potentials, which are not required for transmitter release in these small cells). There are several candidates for the neurotransmitter mediating taste cell–afferent fiber or taste cell–taste cell signaling, including adenosine triphosphate and serotonin.

Highly selective epithelial Na^+ channels (ENaCs) are expressed in some taste cells and may be thought of as receptors for salty taste. Upon stimulation with

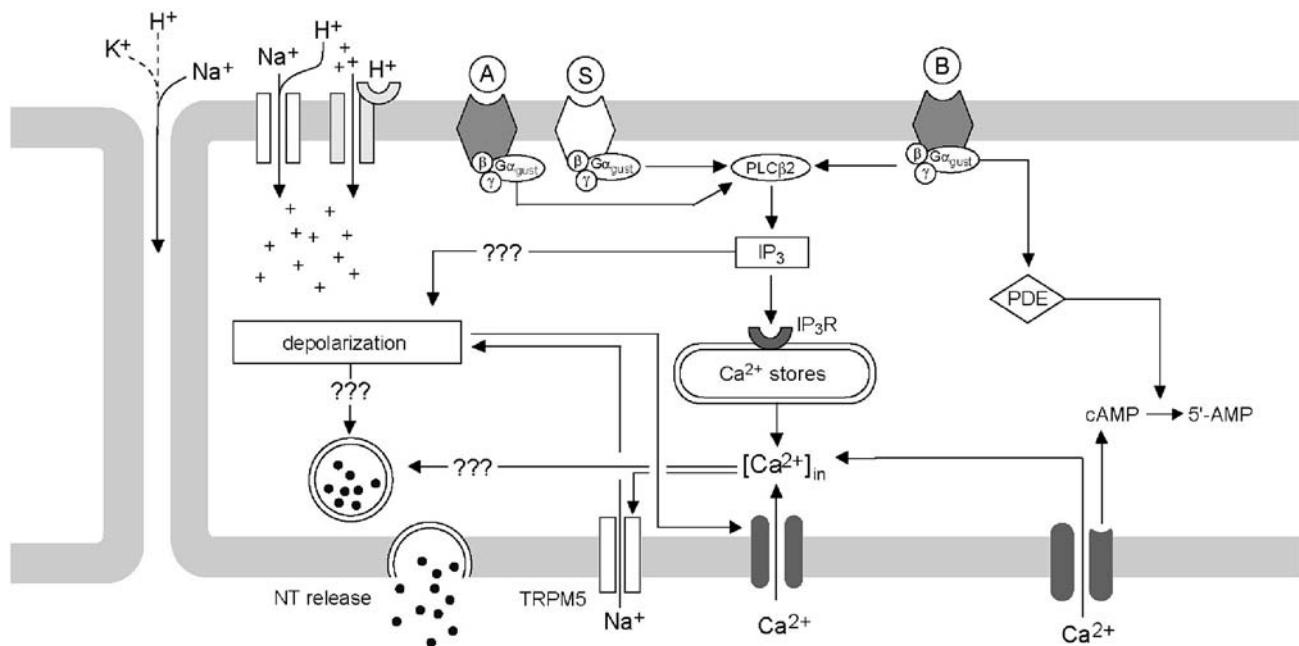


Fig. 3. Taste transduction mechanisms. Sodium salt transduction involves the passage of Na^+ into the receptor cell through passive, amiloride-blockable ion channels on the apical membrane (*top*); protons (H^+) can also penetrate this pathway. In addition, Na^+ , and probably other cations (such as K^+) and protons, can pass through the tight junctions between cells in the taste bud to interact with basolateral ion channels. Acids also gate apical ion channels, which allows entry of cations. These ionic mechanisms lead to direct depolarization of the taste receptor cell. The transduction of sweet (S) and amino acid–tasting (umami) compounds (A) involves membrane-bound G protein–coupled receptors (GPCRs) that bind to sugars or artificial sweeteners, or to amino acids. Bitter substances (B) bind to a different family of GPCRs. GPCRs recognizing each of these types of stimuli couple to the G protein gustducin, which in turn stimulates a phospholipase (PLC- β 2). Activation of PLC- β 2 leads to an increase in intracellular calcium, mediated by inositol triphosphate (IP_3) –dependent release from internal stores, or via voltage-gated basolateral calcium channels. Bitter stimulation may lead to the activation of a cyclic nucleotide–gated ion channel. In all cases, the increase in intracellular calcium likely affects a recently discovered element in the transduction cascade, a transient receptor protein channel (TRPM5). This channel has been directly implicated in the transduction of sweet, amino acid, and bitter taste. Cations permeate this channel and contribute to depolarization. Ultimately, the rise in intracellular calcium and/or depolarization of the taste cell leads to neurotransmitter (NT) release, although the exact mechanisms behind this release have not been fully characterized.

NaCl , Na^+ ions enter taste cells through ENaCs, resulting in depolarization. These channels, like similar sodium channels of the kidney, can be blocked with the diuretic compound amiloride. NaCl also produces a response in taste receptor cells that is not attenuated by amiloride. This mechanism may involve the ability of cations to diffuse through tight junctions between receptor cells in an anion-dependent pathway. Recent evidence also suggests the involvement of an apically located nonselective cation channel. The transduction of nonsodium salts, such as potassium chloride (KCl) or ammonium chloride (NH_4Cl), which are characterized by multiple taste qualities, is not completely understood, but likely depends on a nonselective cation channel. Sour taste stimuli (i.e., H^+) also depolarize taste cells directly, either by permeating or gating apical cation channels.

Sweet, umami, and bitter-tasting compounds interact directly with apically located GPCRs. Mammals possess a family of three receptor genes implicated in sweet and umami taste that encode three distinct subunits, termed T1R1, T1R2, and T1R3. A heterodimer consisting of T1R2 + T1R3 comprises a functional sweet taste receptor that responds in a broad fashion to most sugars and sweeteners. A second heterodimer consisting of T1R1 + T1R3 is likewise a functional receptor for umami stimuli (i.e., L-type amino acids). For bitter-tasting stimuli, there is a family of about 16 to 37 genes in mammals (depending on species; there are 28 in humans) encoding receptor proteins called T2Rs. Unlike the sweet and umami taste receptors, these bitter taste T2R receptors appear to possess a narrow ligand sensitivity, with receptors responding to just one or a few stimuli (although only a handful of T2R receptors have been characterized). Allelic variation of one human bitter taste receptor gene, *TAS2R38*, is largely responsible for differentiating people as “tasters” or “nontasters” of PTC. This variation has been shown to have consequences for dietary selection, as PTC tasters may be more likely to avoid certain cruciferous vegetables such as broccoli, which are rich in thiourea-containing compounds.

Interestingly, although sweet, umami, and bitter GPCRs are all expressed in separate populations of type II taste receptor cells, they appear to share a common second-messenger cascade. Stimulus binding to these receptors activates a taste-specific G protein, gustducin, which in turn stimulates a phospholipase (PLC- β 2). Activation of PLC- β 2 leads to the gating of the transient receptor protein channel TRPM5, which mediates calcium entry into the cell,

in turn facilitating transmitter release onto neighboring cells or afferent fibers.

2.4. Peripheral Innervation

Taste receptors are innervated by branches of the seventh (i.e., facial), ninth (i.e., glossopharyngeal), and tenth (i.e., vagus) cranial nerves. These special visceral afferent fibers project centrally into the rostral pole of the nucleus of the solitary tract (NST), which is the rostral-most extension of the visceral afferent column in the medulla. Taste buds in the fungiform papillae on the anterior portion of the tongue are innervated by the chorda tympani branch of the facial nerve, and those on the soft palate are innervated by its greater superficial petrosal branch. The cell bodies of these fibers are located in the geniculate ganglion and project to the most rostral extension of the NST. Circumvallate and most foliate taste buds are supplied by the lingual-tonsillar branch of the glossopharyngeal nerve, although the most rostral foliate taste buds are innervated by the chorda tympani nerve. Afferent fibers of the glossopharyngeal nerve project through the inferior glossopharyngeal (i.e., petrosal) ganglion to the NST just caudal to, but overlapping with, the facial nerve termination. The pharyngeal branch of the glossopharyngeal nerve innervates taste buds in the nasopharynx. Taste buds on the epiglottis, aryepiglottal folds, and esophagus are innervated by the internal portion of the superior laryngeal nerve, which is a branch of the vagus nerve. Afferent fibers of the superior laryngeal nerve project by means of their cell bodies in the inferior vagal (i.e., nodose) ganglion to the NST caudal to, but overlapping with, the glossopharyngeal nerve termination.

2.5. Afferent Signaling of Taste Information

The gustatory nerves are mixed nerves containing afferents of both the gustatory and somatosensory systems as well as parasympathetic efferents innervating major and minor salivary glands. Single axons that innervate taste buds usually branch to contact several taste receptor cells (which need not be localized to the same bud), which may in part account for their broad tuning. That is, single axons may respond to multiple compounds, even those that elicit distinct tastes (e.g., sucrose and NaCl). Indeed, single axons may also respond to both taste and temperature stimulation. This has led many researchers to propose that the quality of taste is represented by a population code, that is, by the relative activity of many (or all)

taste neurons, rather than by a labeled line code, that is, the activity of a specific subset of taste neurons.

The idea that many neurons together represent a particular taste seems to contradict the notion that humans sense different tastes in different parts of the tongue (e.g., bitter in the back, sweet on the tip, sour on the sides). It has been known for quite some time, however, that humans can discriminate different tastes (e.g., sweet from salty) even when stimulation is limited to a single papilla. In fact, although some slight differences in sensitivity to various taste compounds exist in different parts of the oral cavity, no one region of the oral cavity is specialized to detect any particular type of taste.

Why, then, do humans have taste buds distributed in different parts of the oropharynx and esophagus? Stimulation of the anterior lingual receptors occurs during food sampling and chewing, whereas stimulation of posterior lingual and palatal receptors occurs during swallowing. Certain taste receptors (such as those on the epiglottis) may not interact with tastants at all during normal ingestion but may be stimulated when foodstuffs have been aspirated into the trachea. Stimulation arising from different parts of the oral cavity may therefore be differentially relevant to tastant identification, to the control of chewing, swallowing, and rejection reflexes, or to initiation of reflexes to remove foodstuffs from the airways.

3. CENTRAL TASTE SYSTEM

3.1. Central Taste Pathways

Secondary gustatory fibers arise from the NST to project rostrally more or less parallel to the projection of general visceral sensation, which arises from the more caudal aspects of the solitary nucleus. In most mammalian species, there is a second-order projection into the parabrachial nuclei (PbN) in the pons, from which third-order fibers arise to project ipsilaterally through a classic sensory path to the parvicellular division of the ventroposteromedial nucleus (VPMpc) of the thalamus and then to the agranular insular cortex (Fig. 4).

In addition to the thalamocortical projection, fibers travel from the pons, along with other visceral afferent fibers, along a limbic pathway into areas of the ventral forebrain involved in feeding and autonomic regulation, including the lateral hypothalamus, the central nucleus of the amygdala, and the bed nucleus of the stria terminalis (Fig. 4). In the monkey, taste fibers bypass the pontine relay and project ipsilaterally through the central tegmental

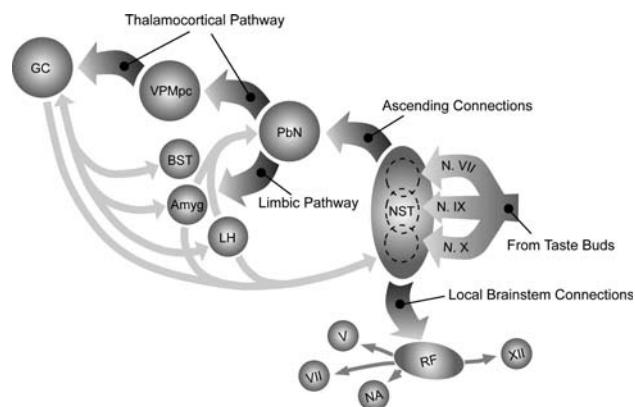


Fig. 4. Mammalian gustatory afferent pathway. Peripheral fibers with different degrees of responsiveness to taste stimuli project into the nucleus of the solitary tract (NST). Fibers of cranial nerves VII, IX, and X project in an organized, overlapping termination within the rostral portion of the NST. Second-order cells project into the parabrachial nuclei (PbN) of the pons, from which a classic *thalamocortical pathway* proceeds to the parvicellular division of the ventroposteromedial nucleus (VPMpc) of the thalamus and then to the gustatory cortex, located within the agranular insular cortex of rodents. Another projection, the *limbic pathway*, arises from the PbN to connect to areas of the ventral forebrain involved in the control of feeding and in autonomic regulation, including the lateral hypothalamus (LH), the bed nucleus of the stria terminalis (BST), and the central nucleus of the amygdala. Cells of the NST also make local reflex connections through the reticular formation (RF) with cranial motor nuclei (trigeminal, V; facial, VII; nucleus ambiguus, NA; hypoglossal, XII) that control muscles involved in facial expression, licking, chewing, and swallowing. In primates, cells of the NST bypass the PbN and project directly to the thalamus (not shown). Many of the forebrain targets of the gustatory system send descending projections back to the brain-stem nuclei (PbN and NST).

tract directly to the VPMpc in the thalamus. The VPMpc projects in the primate to the insular and opercular cortex and to area 3b on the lateral convexity of the precentral gyrus. What may be a secondary cortical area, receiving input from the anterior insula, is found within the posterior orbitofrontal cortex. None of these cortical areas are purely gustatory, because they all contain neurons that are responsive to other sensory modalities such as touch and temperature. There are few studies of the anatomy of these projections in humans, although a careful review of the clinical literature suggests that the human gustatory system is very similar to that of the Old World monkey. A schematic of the probable gustatory projections in humans is shown in Fig. 5.

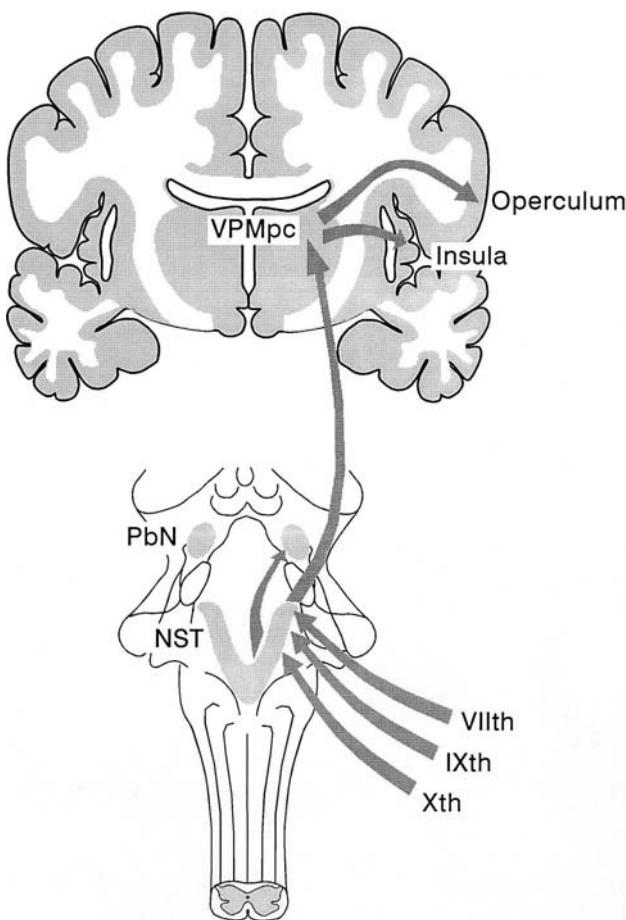


Fig. 5. Drawing of the probable gustatory afferent pathway in humans. Fibers of cranial nerves VII, IX, and X project into the nucleus of the solitary tract (NST), from which there is a direct projection to the parvicellular division of the ventro-posterior medial nucleus (VPMpc) of the ipsilateral thalamus. From the thalamus, fibers project into the insular cortex and frontal operculum. Visceral afferent fibers from the caudal NST project to the parabrachial nuclei (PbN), which also receives gustatory projections in non-primates. In addition to this classic sensory projection, there are numerous subcortical projections of the gustatory nuclei in a variety of species (see Fig. 4).

3.2. Coding of Gustatory Quality

Electrophysiologic recordings from the brain-stem taste areas show that central gustatory neurons are even more broadly tuned than are those in the periphery. Most neurons respond to several stimuli, even those that taste different (e.g., sucrose and NaCl). Taste neurons throughout the gustatory neuraxis respond to tactile and temperature stimulation as well. Because taste cells in central taste nuclei are broadly tuned across stimulus qualities and are also modulated by stimulus concentration, the activity in any one cell cannot unambiguously signal either

quality or intensity. Similarities and differences in patterns of activity across all neurons can be quantified by calculating the correlations among the responses evoked by a series of chemical stimuli across a sample of taste neurons (Fig. 6). These

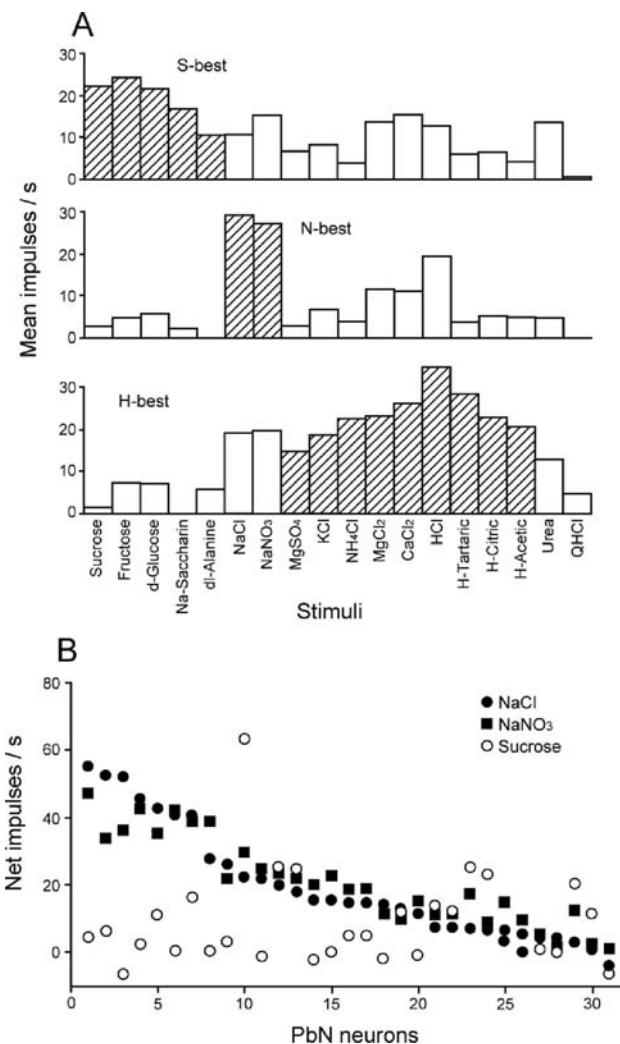


Fig. 6. Broad tuning of gustatory neurons and its relation to patterns of neuronal activity. (A) Mean responses of each of three neuron classes in the hamster PbN to 18 stimuli. The sweet-tasting stimuli are shaded in the profile for the sucrose (S)-best cells, the sodium salts are shaded in the profile for the NaCl (N)-best neurons, and the nonsodium salts and acids are shaded in the response profile for the HCl (H)-best neurons. (Data from Smith DV, Van Buskirk RL, Travers JB, Bieber, SL J Neurophysiol 1983;50:541.) (B) Patterns of activity generated across the hamster PbN neurons depicted in (A) by two sodium salts (*filled symbols*) and by sucrose (*open symbols*). The across-neuron patterns evoked by NaCl and NaNO₃ correlated +0.94, whereas that to sucrose was not correlated with the pattern evoked by either sodium salt (e.g., $r = -0.09$ with NaCl). Stimuli with similar taste produce highly similar responses across the entire population of taste-responsive neurons.

correlations can then be subjected to a multivariate analysis to create a *taste space*, which represents the neurophysiologic similarities and dissimilarities among the responses to the stimuli across the neuronal population. An across-neuron taste space generated from the responses of cells in the PbN to several stimuli is shown in Fig. 7. The positions of the stimuli in this space reflect similarities and differences in the population response of the gustatory system to these compounds. Stimuli with similar tastes are grouped together, and those with different tastes are separated within this space. The similarities and differences in the patterns of activity evoked by these stimuli provide a basis for their discrimination.

Recent simultaneous recordings from multiple neurons in the gustatory forebrain and cortex has reinforced the notion that the representation of a taste dynamically changes through time. For example,

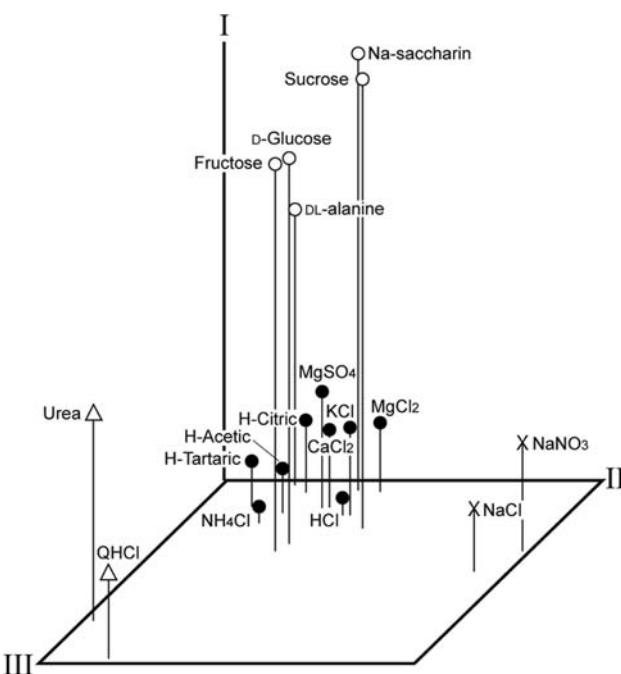


Fig. 7. Three-dimensional *taste space*, showing the similarities and differences in the across-neuron patterns evoked by 18 stimuli delivered to the anterior tongue of the hamster. This space was derived from multidimensional scaling of the across-neuron correlations among these stimuli recorded from neurons in the parabrachial nuclei of the hamster. The proximity of stimuli within this space indicates a high degree of correlation between the across-neuron patterns elicited by these compounds. Four groups of stimuli are indicated by different symbols: sweeteners, sodium salts, nonsodium salts and acids, and bitter-tasting stimuli. Stimuli with similar taste produce highly correlated patterns of activity. (Data from Smith DV, Van Buskirk RL, Travers JB, Bieber SL J Neurophysiol 1983;30:54 l.)

gustatory cortex neuronal activity represents first tactile information, then taste quality information, and finally hedonic information. Taste information is essentially multiplexed such that single neurons participate in representing multiple features of a stimulus.

3.3. Descending Modulation of Taste Neural Activity

Responses of brain-stem cells to gustatory stimulation are subject to several modulatory influences. For example, glucose, insulin, and pancreatic glucagon, when systemically administered, alter the responses of cells in the rat NST to tongue stimulation with glucose. Taste-evoked responses are also modified by alterations of the body's fluid homeostasis, including restriction of sodium intake, administration of diuretics, or elevation of levels of renal or adrenal hormones (such as renin and aldosterone). Conditioned taste-aversion learning shifts the patterns of response to taste stimuli recorded from the rat NST.

The precise mechanisms underlying these effects are unknown. As is clear from Fig. 4, however, the taste system is a broad neural network in which the interaction of dozens of brain areas continually shape neural responses. Even in anesthetized animals, it is known that the direct descending projections from gustatory areas of the ventral forebrain and cortex to both PbN and NST (Fig. 4) are physiologically relevant. Inputs from the gustatory cortex, the central nucleus of the amygdala, and the lateral hypothalamus have all been shown to both excite and inhibit the activity of cells of the rostral NST evoked by oral application of gustatory stimuli.

Recent studies have begun to reveal mechanisms of synaptic transmission within the gustatory region of the NST. It is very likely that glutamate acts as a neurotransmitter between gustatory afferent fibers and taste-responsive cells in the NST. Electrophysiologic recordings from cells in the rostral NST from both rats and hamsters have shown that gamma-aminobutyric acid (GABA) produces inhibition of activity in these cells, which is mediated predominately by the GABA_A receptor subtype. One of the roles of GABA may be to regulate the breadth of tuning of these cells, which respond to more taste stimuli when GABA activity is blocked by bicuculline. Evidence also indicates that taste-responsive cells in the NST are excited by the neurotransmitter substance P (SP) and inhibited by met-enkephalin, an opioid peptide.

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Michael T. Shipley, Matthew Ennis and Adam C. Puche

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1. INTRODUCTION

Transduction of olfactory information occurs when odorant molecules contact the dendrites of olfactory receptor neurons (ORNs). These neurons reside in the olfactory epithelium, a specialized region of the dorsal nasal cavity. ORN axons project through the lamina propria underlying the olfactory epithelium and into the glomerular layer of the main olfactory bulb. This projection forms the olfactory nerve, or cranial nerve I. Within glomeruli, ORN axons synapse onto the apical dendrites of mitral and tufted cells, which are the output neurons of the main olfactory bulb (MOB). In turn, axons from these cells project to the primary olfactory cortex, via the lateral olfactory tract. The primary olfactory cortex comprises several brain regions, including the anterior olfactory nucleus, the piriform cortex, parts of the amygdala, and the entorhinal cortex. These areas, in turn, are interconnected with many areas of the brain, including the neocortex, hippocampus, mediodorsal thalamus, preoptic area, hypothalamus, and other parts of the limbic system. Through these connections, the olfactory system influences a wide range of behaviors and physiologic functions including reproduction, social behavior and communication (e.g., scent marking), food finding and selection, and maternal behavior in addition to regulating neuroendocrine functions.

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We are only beginning to understand how odors are “coded” by primary olfactory neurons in the nasal epithelium and by activity patterns in the MOB. Recent advances in genetic approaches, electrophysiology, and imaging of neural function promise to rapidly close this critical gap.

2. THE OLFACTORY EPITHELIUM

2.1. Olfactory Receptor Neurons

ORNs are contained in a neuroepithelium located in the dorsocaudal nasal vault, along the upper portion of the nasal septum, the cribriform plate, and the medial wall of the superior turbinate. Sensory information is carried along ORN axons to the brain. Airborne molecules enter the nasal cavity, where they are subject to relatively turbulent air currents. The duration, volume, and velocity of a sniff as well as the composition of the layer of mucus that covers the olfactory epithelium are all important determinants of the effectiveness of an odor (Fig. 1).

ORNs lie in a pseudostratified columnar epithelium, which is thicker than the surrounding respiratory epithelium of the nasal cavity. This epithelium rests on a highly vascular lamina propria. Within the epithelium are the bipolar ORNs, supporting cells (sustentacular cells), microvillar cells, and basal cells; Bowman’s glands lie within the underlying lamina propria. Unlike taste receptor cells, which

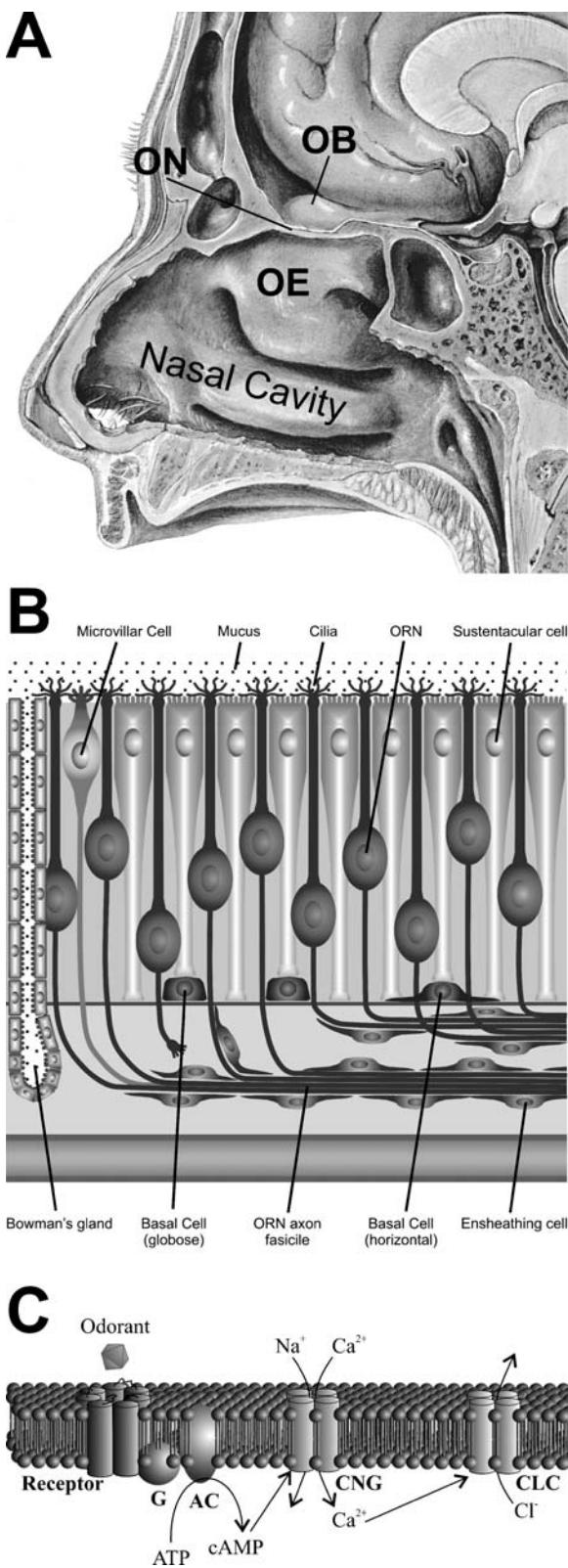


Fig. 1. The olfactory epithelium. (A) Sketch of the lateral view of the human nasal region showing the olfactory epithelium (OE) lining the caudal nasal cavity and part of the brain. Axons from sensory neurons in the OE project through the cribriform plate and form the olfactory nerve (ON), which

are modified epithelial cells, olfactory receptors are true neurons possessing both a dendrite and an axon. Their cell bodies are in the basal two-thirds of the epithelium, and their apical dendrite extends to the epithelial surface where it swells to form the olfactory knob, from which 10 to 12 cilia extend into the mucous layer. These cilia are the primary site of chemosensory transduction. Basal to the ORN cell body, an unmyelinated axon arises and joins other ORN axons. These axons penetrate the basal lamina, at which point they become ensheathed by specialized Schwann cells, the ensheathing cell. These bundles join others to make up the 15 to 20 fascicles (*fila olfactoria*) of the olfactory nerve, which pass through the cribriform plate to synapse in the MOB. These neurons are unique in having direct contact with the external environment at the cilia and with the central nervous system at their axon terminations in the MOB. There is evidence that some viruses and other substances can be incorporated into ORNs and transported along their axons to the MOB. In experimental animal studies, some of these substances escape from ORN synaptic terminals and are incorporated into neurons and axons within the MOB. These substances are then further disseminated to other parts of the brain by anterograde or retrograde axonal transport. Thus, the olfactory nerve is a potential conduit for the entry and spread of foreign substances into the brain. It has been suggested that some neurodegenerative diseases could be the result of viruses, metals or silicates, and so forth, entering the brain from the olfactory epithelium.

Supporting cells of the olfactory epithelium separate and partially wrap ORNs. Approximately one-tenth of the ORNs in humans have microvilli on their apical surface instead of cilia. It is not known if these

terminates in the olfactory bulb (OB). (Modified from Grant's Anatomy MEDICLIP collection [image HNC08021, 1999], Williams & Wilkins, a Waverly Company.) (B) Schematic illustration of the olfactory epithelium showing the major cell types present in the rat epithelium; olfactory receptor neuron, sustentacular cell, microvillar cell, basal cell (globose and horizontal), ensheathing cell, and Bowman's gland cells. (C) Odor molecules bind to specific odorant receptor proteins located in the cilia of ORNs. These 7-TM receptors are coupled to G proteins that activate either adenylate cyclase III (AC) to generate cyclic AMP (cAMP) or putatively phospholipase C (PLC) to generate phosphatidyl inositol (IP₃). These second messengers in turn open channels that admit calcium (Ca²⁺) and/or sodium (Na⁺) into the cilium. These ions lead to membrane depolarization and modulate intracellular free Ca²⁺ levels, both of which lead to the generation of action potentials that are conducted along ORN axons to the olfactory bulb.

microvillar cells have axons contacting the MOB although they appear to be neuronal. Deep to the ORNs, sustentacular and microvillar cells are the basal cells (globose and horizontal). These are stem cells responsible for replacement of ORNs, which in the mouse have a life span of ~40 days. In addition to the typical elements of a lamina propria, the nasal mucosa also contains secretory Bowman's glands, which provide a serous component to the mucous layer covering the olfactory epithelium.

ORNs and their axons contain olfactory marker protein (OMP), which is unique to olfactory neurons and is present in a number of vertebrate species, including humans. OMP is expressed in all ORNs and accounts for approximately 1% of their total protein content. The exact role of this highly abundant protein is poorly understood, but recent evidence suggests that genetic deletion of OMP disrupts olfactory signal transduction, including odor response kinetics, adaptation, and impairs odor perception.

Some chemical stimuli can give rise to activity in the trigeminal system through the stimulation of nerve endings in the nasal and oral cavities. The burning or irritation arising from stimuli like ammonia or hot peppers interacts with olfactory, trigeminal, and taste information in what has been termed the “common chemical sense.” Information about intranasal or intraoral chemical irritation usually remains intact in patients complaining of taste or smell dysfunction and can be useful in the assessment of malingering patients.

2.2. A Large Multigene Family Encodes Odorant Receptors

The structure and number of odorant receptors remained elusive until the 1991 discovery of a large multigene family encoding seven transmembrane (7-TM) receptor molecules expressed in ORNs. For this landmark discovery, Dr. Linda Buck and Dr. Richard Axel were awarded the 2004 Nobel Prize in Physiology or Medicine. In mammals, this family of odorant receptor genes (ORGs) consists of ~1000 separate genes scattered across multiple chromosomes. Remarkably, ORGs comprise 2% to 3% of all expressed genes in the genome. Members of this ORG family have a high degree of base sequence homology with other genes that encode 7-TM receptors that function as neurotransmitter receptors. In humans, up to two-thirds of these ORGs have become pseudo-genes (i.e., genes that have undergone evolutionary mutations rendering them

nonfunctional). In mammals, the typical ORN expresses only a single member of the ORG gene family. ORNs expressing the same ORG are widely dispersed in broad expression zones within the epithelium. Remarkably, however, ORNs expressing the *same* ORG send axons to precisely the same small target areas in the brain. The functional significance is discussed in Section 3.1.

ORGs encode proteins that function as odorant receptors. A large body of evidence including electrical recordings and calcium dye imaging of individual ORNs and the odor response profiles of specific ORGs expressed in heterologous systems indicate that odorant receptors are broadly tuned. A specific receptor may respond optimally (i.e., at low odorant concentration) to a particular structural feature of an odorant (sometimes referred to as an epitope) and to other odors that express the same epitope. It may also respond to higher concentrations of other odors that have different but structurally similar epitopes. Thus, each of the 1000 receptors exhibits a distinct molecular receptive range that has partial overlap with the range of other receptors. Together, these findings imply that an individual odor will activate a distinct pattern of activity that depends on the number of ORN-ORGs activated, which is determined by both odor identity and concentration (see Section 3.1).

2.3. Mechanisms of Olfactory Signal Transduction

The binding of an odor molecule to an odorant receptor triggers a cascade of events culminating in electrical signals sent down ORN axons to the brain. This transduction cascade has been progressively elucidated in the past two decades. Odorant binding to the 7-TM odorant receptors activate an olfactory-specific G protein (G_{olf}), which stimulates adenylate cyclase type III, increasing cAMP production. Early patch clamp studies on excised patches of olfactory cilia demonstrated the expression of a cyclic nucleotide-gated, cation-permeable channel. This olfactory cyclic nucleotide-gated channel (CNGA2) was recently cloned and together with the G_{olf} G protein is a critical element of the odor transduction cascade. Increased cAMP opens CNGA2 channels, which are nonselective cation channels with a preferential permeability for calcium (Ca^{2+}). Ca^{2+} influx via these channels in turn leads to opening of a Ca^{2+} -activated chloride channel, which further depolarizes the cell (due to high intracellular Cl^- levels relative to the mucus), leading to generation

of action potentials that propagate down the axon to the MOB. Genetic null mutations for Golf, CNGA2 channels, or adenylate cyclase III firmly establish the essential role for these molecules in odor transduction. Mice with the null mutations for any of these three critical transduction elements are functionally anosmic.

In the presence of a persistent odor in the environment, the olfactory system undergoes adaptation, reducing the perception of that odor. Much of this perceptual adaptation occurs within ORNs by negative regulation of the signal transduction process. Elevated intracellular Ca^{2+} in an ORN during response to an odor is a key trigger for olfactory adaptation, as evidenced by reduced adaptation when intracellular Ca^{2+} is chelated by 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA). The rise in intracellular Ca^{2+} activates calmodulin, which binds to cyclic nucleotide-gated channels decreasing their affinity for cAMP. Ca^{2+} -calmodulin can also activate CaM kinase II, leading to phosphorylation of both ACIII, reduced production of cAMP, and phosphodiesterase, increasing inactivation of cAMP. These processes are relatively rapid resulting in odor adaptation requiring only seconds to minutes.

2.4. The Olfactory Nerve Has the Capacity to "Replace" Itself

ORNs undergo constant turnover throughout life. Damage to the olfactory nerve results in retrograde degeneration of ORNs, which are then reconstituted from the stem cell population located in the basal epithelium. These new ORNs mature and send axons to the MOB where they form new synaptic connections. The olfactory epithelium may have the capacity to replace itself because its direct exposure to airborne molecules makes it vulnerable to injury from environmental factors such as toxicant exposure, viral infection, and other conditions that injure the olfactory receptor sheet. The high level of newly formed ORNs and axons reestablishing functional synaptic connections with the brain is unique among mammalian primary sensory neurons. Understanding the mechanisms that allow this remarkable neural replacement to take place may eventually provide therapies for promoting repair in other parts of the brain. Indeed, recent studies indicate it may be possible to use the ensheathing cells that surround the olfactory nerve as therapeutic agents for axon regrowth in models of spinal cord injury.

3. THE MAIN OLFACTORY BULB

The MOB is the first site of olfactory information processing in the brain and is an ellipsoid structure that lies on the ventral surface of each frontal lobe dorsal to the cribriform plate of the ethmoid bone. The MOB is arranged in layers, from superficial to deep: the olfactory nerve layer (ONL), the glomerular layer (GL), the external plexiform layer (EPL), the mitral cell layer (MCL), the internal plexiform layer (IPL), the granule cell layer (GCL), and the subependymal layer (SEL).

There are two major neuronal groups in the MOB: second-order neurons (mitral and tufted cells), and interneurons (juxtaglomerular cells, and granule cells). Axons of mitral/tufted (M/T) cells constitute the primary output pathway of the MOB, the lateral olfactory tract (LOT). M/T cell dendrites ramify in two distinct layers of the bulb; their apical dendrites ramify in the glomerular layer and interact with juxtaglomerular cells, and their lateral dendrites extend in the external plexiform layer and interact with granule cells. Thus, the input-output functions of the MOB are critically determined by interactions between M/T, juxtaglomerular cells, and granule cells.

3.1. Glomeruli as Anatomic and Functional Units

ORN axons terminate exclusively in glomeruli, which are spheroid structures composed of a cellular shell surrounding a core of neuropil. Each point within the bulb receives input from ORN neurons widely scattered in the olfactory epithelium. Axons from ORNs expressing the same ORG (i.e., an ORN-ORG cohort) converge and terminate in only two (or a few) glomeruli. Thus, a fundamental principle of functional organization in the olfactory system is that each glomerulus receives sensory input from ORNs that have identical odorant response profiles (Fig. 2).

A variety of approaches, including 2-deoxyglucose incorporation, calcium dyes, intrinsic imaging, and functional magnetic resonance imaging (fMRI) have been used to "map" the response of glomeruli to batteries of odors. These activity maps indicate that specific odor molecules activate multiple glomeruli with overlapping, but different, response specificities that are generally bilateral and consistent across animals of the same species. This is not surprising given that the odorant receptors, which are represented by glomeruli, also show distinct but overlapping odorant specificities. Consistent with the molecular

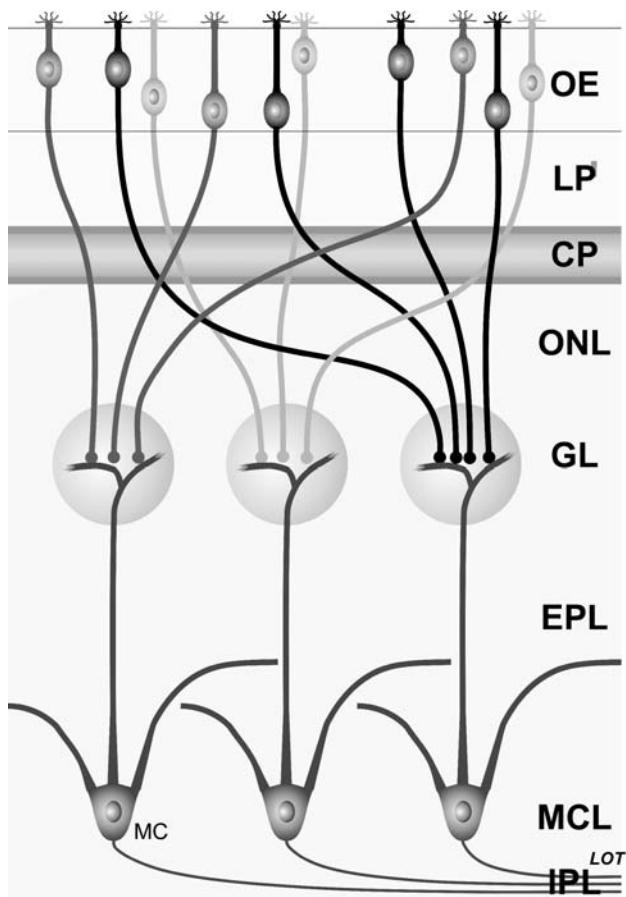


Fig. 2. Convergence of axons from ORNs expressing the same receptor gene. ORNs have a single axon that passes into the lamina propria underlying the olfactory neuroepithelium (OE), through the cribriform plate (CP), and then into the olfactory bulb. These axons synapse on the dendrites of second-order neurons within globular structures of neuropil called glomeruli. ORNs expressing different odorant receptor genes (shown as light, medium, and dark-gray cells) are interspersed randomly within the epithelium. Axons from ORNs expressing the same odorant receptor gene converge and project to a single glomerulus. OE, olfactory epithelium; ONL, olfactory nerve fiber layer; GL, glomerular layer; EPL, external plexiform layer; MCL, mitral cell layer; IPL, internal plexiform layer; MC, mitral cell.

receptive range properties of odorant receptors, increasing the concentration of an odorant increases the number of responsive glomeruli as “weakly tuned” ORN-ORG cohorts are recruited. Structurally similar odorants preferentially activate glomeruli in similar MOB regions, perhaps due to the fact that structurally similar ORN-ORG cohorts often project to similar regions of the MOB. These *modules* or *domains* encompass between 10 and 50 glomeruli. As with the glomeruli themselves, each module is duplicated on the medial and lateral halves of the

bulb and is roughly conserved across individuals. Together these data suggest that, at the level of ORN input, the glomerulus is the smallest detectable unit contributing to the functional representation of odor information and that information about odorant identity is coded by spatiotemporal patterns of glomerular activity.

3.2. Neural Circuits of the Glomerular Layer

The spatial and temporal characteristics of glomerular activity maps are initially determined by the molecular receptive ranges of ORN-ORG cohorts and their precise topographic projections to glomeruli. These characteristics neither involve nor require interactions among ORNs. The next step in olfactory processing, beginning with olfactory nerve (ON) synapses in glomeruli, is dependent upon neuronal interactions in the MOB network. This neural processing involves both the intrinsic properties of these postsynaptic neurons and the synaptic relationships that organize them into neural circuits.

Glomeruli are composed of a “shell” of juxtaglomerular (JG) neurons and astrocytes surrounding a neuropil, which contains the synaptic contacts between ORN axons, the apical dendrites of mitral and tufted cells, the dendrites of JG neurons, and the synaptic linkages among these neurons. Although classic neuroanatomists recognized at least three types of JG neurons—periglomerular (PG), external tufted (ET), and short axon (SA)—there has been a tendency to view the glomerulus as composed entirely of PG cells, local GABAergic interneurons that function to negatively modulate ORN to mitral/tufted (M/T) cell synaptic throughput. Recent research indicates that this view is too simplistic and shows that each glomerulus contains complex micronetworks that perform multiple, distinct operations on ORN input. Based on new information of the major synaptic relationships among ET, PG, and SA neurons, at least four principle glomerular circuits can be postulated: (1) the ON → M/T → PG circuit, (2) the ON → PG circuit, (3) the ON → ET → PG circuit, and (4) the ON → ET → SA interglomerular circuit (Fig. 3).

1. *The ON → M/T → PG circuit:* ON terminals synapse on the apical intraglomerular dendrites of M/T cells. Classic EM studies indicate that many PG cell synapses are with M/T cell intraglomerular dendrites that are thus *presynaptic* to PG cells forming the ON → M/T → PG circuit. By this circuit, ON-evoked excitation of M/T cells causes M/T excitation of PG cells, which provide feedback inhibition of M/T cells.

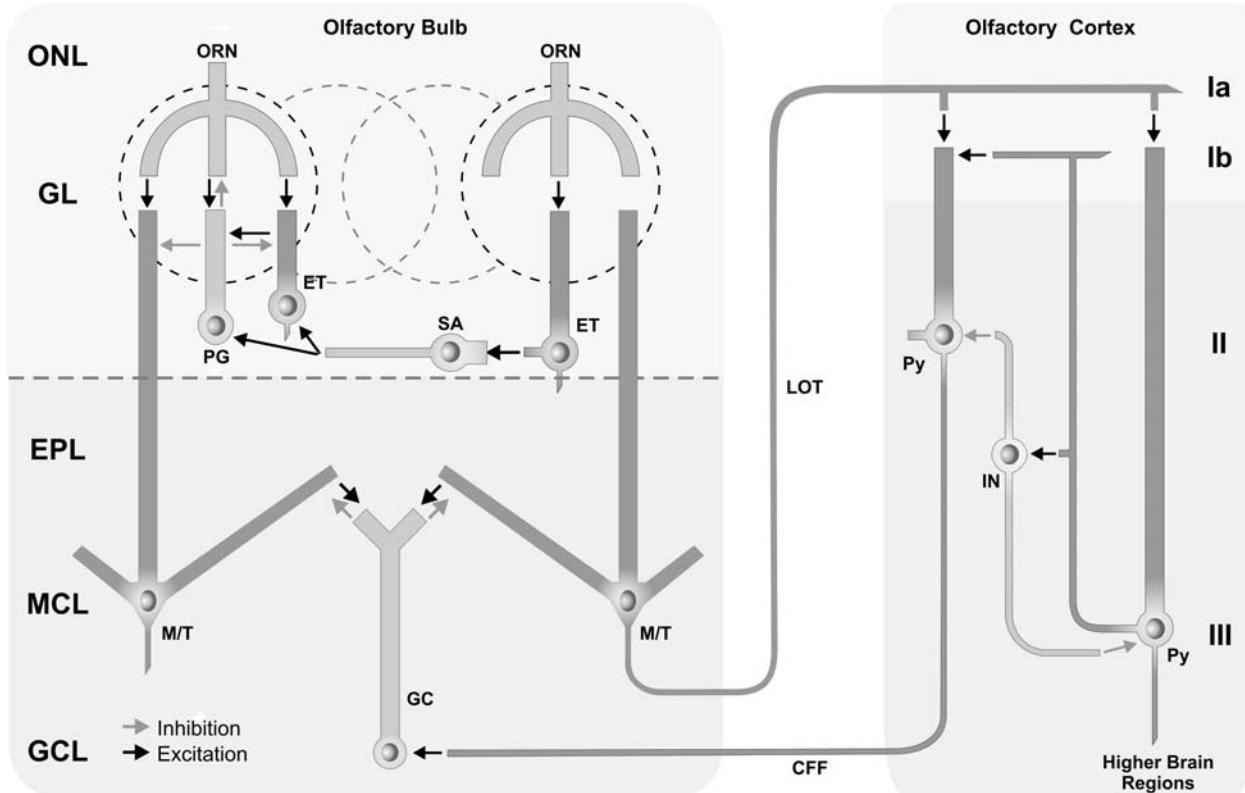


Fig. 3. Basic circuitry of the main olfactory bulb and olfactory cortex. The principle circuits of the main olfactory bulb include the ON→M/T→PG, ON→PG, ON→ET→PG, ON→ET→SA→PG→M/T, and MT→GC circuits. Mitral/tufted cell axons exit the caudal bulb forming the lateral olfactory tract (LOT) and terminate in layer Ia of the cortex on the dendrites of layer II to III pyramidal (Py) cells. Pyramidal cells project axons to other cortical regions terminating in layer Ib (the association fibers) and back to the olfactory bulb (centrifugal fibers) and to higher brain regions. Interneurons in the cortex generate both feedback and feedforward inhibition. ONL, olfactory nerve fiber layer; GL, glomerular layer; EPL, external plexiform layer; MCL, mitral cell layer; IPL, internal plexiform layer; GCL, granule cell layer; ORN, olfactory receptor neuron; PG, periglomerular neuron; ET, external tufted cell; SA, short axon cell; M/T, mitral/tufted cell; GC, granule cell; LOT, lateral olfactory tract; CFF, centrifugal fiber tract; Py, pyramidal cell; IN, cortical interneuron.

2. *The ON → PG cell circuit:* PG cells also receive ON synaptic contacts suggesting that they could provide feed-forward inhibitory input to M/T cells via an ON→PG→M/T circuit. Another established role for the ON→PG circuit is presynaptic inhibition of ON terminals, via an ON→PG→ON circuit. Feedback from PG cells onto ORN axon terminals suppresses transmitter release (i.e., pre-synaptic inhibition). Both GABA and dopamine are involved in this inhibition. ORNs express GABA_B and dopamine D₂ receptors on their axon terminals, and both GABA_B and D₂ agonists suppress postsynaptic activity evoked by ON impulses.

3. *The ON → ET → PG circuit:* The ON provides monosynaptic input to all ET cells, which, in turn, monosynaptically excite PG cells. ON-evoked excitation of ET cells is followed by longer latency ET cell inhibition, due to GABAergic

input from PG cells. Thus, one extension of the ON→ET→PG circuit is an ON→ET→PG→ET feedback circuit. The temporal sequence of excitation followed by feedback inhibition generated by this circuit may function to shape postsynaptic responses to sensory input possibly by creating a temporal “window” for sensory signals to influence glomerular output. The ON→ET→PG circuit may also provide feed-forward inhibition to M/T cells forming an ON→ET→PG→M/T circuit.

4. *The ON → ET → SA interglomerular circuit:* By contrast with ET and PG cells, SA cells have dendrites that contact several glomeruli and have axons that contact numerous glomeruli over a spatial extent of 1 mm. The existence of this interglomerular network suggested that SA cells mediate interactions among glomeruli. SA cells lack monosynaptic ON input but do receive excitatory

input from ET cells. SA cells make excitatory (glutamatergic) synapses with PG and ET cells in distant glomeruli, and activation of interglomerular axons results in GABA_A receptor-mediated inhibition in mitral cells in distant glomeruli. Thus, the ON→ET→SA→PG→M/T cell circuit provides interglomerular lateral inhibition, which may enhance contrast among differentially activated glomeruli. Because SA axons also excite ET cells and ET cells excite additional local SA cells, activity can potentially propagate throughout the entire population of glomeruli. Modeling studies have proposed that as odor concentration increases, the interglomerular network might “normalize” postsynaptic activity by inhibiting mitral cells in concentration-dependent manner.

3.3. Infraglomerular Circuits

M/T cells extend four to six lateral dendrites for considerable distances (1 to 2 mm in mouse) through the external plexiform layer (EPL), deep to the glomerular layer. M/T dendrites interact with the apical dendrites of the GABAergic granule cells (GCs), whose cell body resides in the mitral and granule cell layers. GC and M/T cell dendrites form dendrodendritic synapses: M/T cells make excitatory synapses onto GCs, and GCs make inhibitory synapses onto M/T cells, thus forming an M/T↔GC circuit. These dendrodendritic synapses function to provide feedback and lateral (feedforward) inhibition.

In addition to the dendrodendritic contacts, GCs are heavily targeted by excitatory synaptic inputs from ipsi- and contralateral olfactory cortical structures via the centrifugal afferents. Excitatory inputs onto GCs induce release of GABA onto M/T cell lateral dendrites, thus inhibiting M/T cells. The cortical regions targeting GCs are themselves targeted by the M/T cells. Therefore, output from the MOB can cause a negative feedback to the output M/T cells via centrifugal afferents and the A GC→M/T circuit. The functional significance of this circuitry is poorly understood, but it would appear that the coding of olfactory information involves feedback mechanisms in which the output of the bulb at any moment is fed back to the bulb to modify its subsequent outputs. The cell bodies of some GCs are also coupled by gap junctions, which could serve to electrically couple clusters of GCs such that synaptic activation of one cell leads to the activation of other cells in the cluster. This arrangement could “amplify” or “synchronize” granule cell inhibition of M/T lateral dendrites.

There are additional, poorly understood classes of interneurons present in the granule cell layer. Recently, findings show that one of these cell types, the Blanes cell (BC), fires action potentials for up to 15 min after brief excitation. As BCs inhibit GCs, excitation of BCs may produce prolonged epochs of GC inhibition. This, in turn, would decrease GC-mediated inhibition of M/T cells (i.e., disinhibition of M/T cells). The functional role of the BC is unclear but may act to “disconnect” the M/T cell from feedback/feedforward inhibition from M/T or cortical inputs.

3.4. Mitral Cell Responses to Odor Stimulation

There have been relatively few electrophysiologic studies of the responses of MOB neurons to odors *in vivo*. These responses are typically complex. Extracellular recordings show that mitral cells may be initially excited then inhibited, inhibited then excited, or may exhibit more complex responses. Because mitral cell odor responses are strongly modulated by postsynaptic processing, it has been difficult to determine the direct roles of odor identity and concentration in M/T responses. Early studies suggested that M/T cells respond differently to at least two odors at all odor concentrations. Similar results in the salamander led to the concept of *concentration tuning* of mitral cell: individual cells appeared to respond best to a particular concentration of each odorant. More recent work indicates that, similar to ORNs expressing a specific receptor, M/T cells exhibit a distinct molecular receptive range, responding optimally to a group of structurally similar odors at low concentrations. Additionally, most electrophysiologic studies of bulb neurons have been done in anesthetized animals. Studies in unanesthetized animals indicate that M/T cell odor responses may be more narrowly tuned than previously suspected and strongly dependent on behavioral context.

Clearly, understanding of the neural correlates of odor perception lags behind knowledge of the stimulus-response characteristics of neurons in other sensory systems. The preponderance of evidence suggests that odor quality and concentration are not uniquely represented by single neurons in a labeled-line code but rather by the activity of glomerular populations or ensembles of cells. Another important element of olfactory coding may include temporal patterns of activity, which is dependent upon sniffing frequency and oscillations within the MOB network. Investigation of these and other potential coding mechanisms will require the use of experimental

techniques to monitor activity from large numbers of neurons combined with behavioral measures of olfactory perception.

3.5. Modulatory Inputs to MOB

MOB also receives extrinsic inputs from cholinergic, noradrenergic, and serotonergic cell groups in the basal forebrain and brain stem. Cholinergic (and GABAergic) projections arise primarily from the nucleus of the horizontal limb of the diagonal band nucleus. Noradrenergic projections arise from the pontine nucleus locus coeruleus. The midbrain dorsal and median raphe provides strong serotonergic inputs to the MOB. Each of these projections terminates broadly across most layers of the MOB, with the exception of the ON layer. Behavioral studies have shown that these neuromodulatory inputs are

important for odor discrimination and/or the formation of odor memories.

4. THE PRIMARY OLFACTORY CORTEX (FIG. 4)

4.1. Anterior Olfactory Nucleus

The MOB is connected to the anteroventral pole of the temporal lobe by a stalk of tissue called the olfactory peduncle, which consists of a population of neurons, the anterior olfactory nucleus (AON), and two major tracts of fibers, the lateral olfactory tract (LOT) and the anterior limb of the anterior commissure. Though historically referred to as a “nucleus,” the AON is now considered a cortical structure with several subdivisions distinguished on the basis of cellular architecture and connectional patterns. A

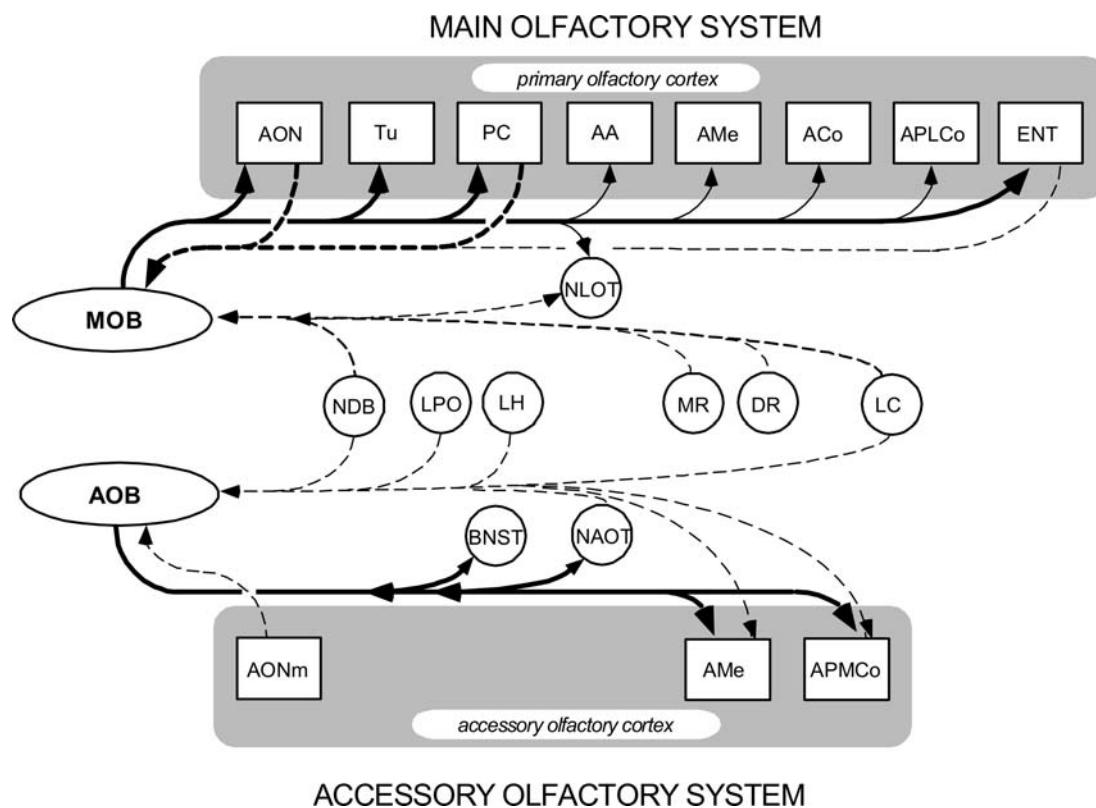


Fig. 4. Major connections of the main and accessory olfactory systems. Diagram showing the main and accessory olfactory bulb connections with cortical (gray panels) and subcortical (circles) structures. Relative strengths of connections are represented by line thickness. Output projections of MOB and AOB are shown by solid lines, and the reciprocal and centrifugal projections to MOB and AOB are shown by dashed lines. Cortical areas composing the primary and accessory olfactory cortex are indicated by squares. AA, anterior amygdala; AOB, accessory olfactory bulb; ACo, anterior cortical amygdaloid nucleus; AMe, medial amygdaloid nucleus; AON, anterior olfactory nucleus (m, medial division); APLCo, posterolateral cortical amygdaloid nucleus; APMCo, posteromedial cortical amygdaloid nucleus; BNST, bed nucleus of the stria terminalis; DR, dorsal raphe nucleus; ENT, entorhinal cortex; LH, lateral hypothalamus; LC, locus coeruleus; LPO, lateral preoptic area; MOB, main olfactory bulb; MR, median raphe; NAOT, nucleus of the accessory olfactory tract; NLOT, nucleus of the lateral olfactory tract; NDB, nucleus of the diagonal band; PC, piriform cortex; Tu, olfactory tubercle.

substantial number of AON neurons are pyramidal cells whose apical dendrites extend toward the pial surface of the AON.

Major afferents to AON arise from the ipsilateral bulb, the contralateral AON, from more caudal primary olfactory cortical structures, and from a variety of cortical and subcortical structures associated with the limbic system. The major efferents of the AON are to (i) the *ipsilateral* bulb and olfactory cortex and (ii) the *contralateral* bulb and AON. Knowledge of the functional significance of AON is rudimentary. The major interbulbar connections of the AON clearly implicate this structure in the interhemispheric processing of olfactory information. There is evidence that binasal mechanisms may function in spatial localization of odors, and the AON system would be suspected to play a significant role in such mechanisms. There is also evidence from animal studies that the AON plays a key role in the interhemispheric transfer of olfactory memories.

4.2. Piriform, Periamygdaloid, and Parahippocampal Cortex

At the caudal limits of the AON, the basal temporal lobe expands in the caudal direction forming a pear-shaped structure, the piriform lobe. The rostral part of this structure contains the piriform cortex, the major cortical component of the primary olfactory cortex. Further caudal, the medial part of this cortex overlies the amygdaloid complex, and this part of the piriform cortex is referred to as the periamygdaloid cortex. Continuing caudally and medially, the piriform-periamygdaloid cortex gives way gradually to the parahippocampal cortex and then the hippocampus. Thus, there is a continuous expanse of gradually changing cortical architecture leading from the MOB, the AON, piriform, periamygdaloid, entorhinal, and subicular cortices and directly into the fields of the hippocampus. This orderly anatomic arrangement is one reason that early anatomists referred to the olfactory cortex, parahippocampus, and hippocampus as the “rhinencephalon,” believing that the entire expanse of cortex constituted the “smell brain.” This view was tempered by subsequent research showing that the projections of the MOB directly innervate only the AON, piriform, periamygdaloid, and lateral entorhinal cortices. Thus, most of the parahippocampal region and the hippocampus are now considered to be part of the *limbic system*. Notwithstanding this considerable loss of cortical real estate, the olfactory system can still claim to be

the sensory system with the most direct access to the hippocampus, because there are direct projections of the MOB and piriform cortex to the entorhinal cortex, which is a major source of afferent input to the hippocampus. Collectively, the AON, piriform, periamygdaloid, and parts of the parahippocampal cortex are referred to as *primary olfactory cortex (POC)* as they are all directly targeted by synaptic inputs from the MOB.

4.3. Connections of Primary Olfactory Cortex

The piriform cortex (PC) is a three-layer cortical structure subdivided on the basis of cytoarchitecture and afferent connections. Layer I, the most superficial plexiform layer, is divided into Ia, which receives afferents from the ipsilateral MOB via the lateral olfactory tract (LOT), and Ib, which receives afferents from pyramidal neurons in different parts of the POC (also referred to as association fibers). Layer II contains the highest cell density, whereas layer III has a lower cell density but is the thickest layer. Neurons in PC are divided into two main classes: principal or output neurons (pyramidal cells) and intrinsic interneurons. Superficial pyramidal cells are located in layer II and deep pyramidal cells in layer III. Both types of pyramidal cells share characteristic features, including (1) a primary apical dendrite that extends radially toward the surface and arborizes in layer Ia and Ib; (2) secondary or basal dendrites that extend from the soma into deeper layers of PC; and (3) a myelinated axon that typically extends deep to the soma terminating on other local pyramidal cells and interneurons (*see later*) or projecting back to MOB. Pyramidal cells are glutamatergic and participate in the intrinsic and extrinsic (association) excitatory projections discussed below. The PC contains a wide variety of interneurons classified by morphologic and neurochemical expression distributed throughout all layers and regions of this structure, but most, if not all, contain GABA. These GABAergic inhibitory neurons provide feedback and feedforward inhibition of pyramidal cells.

The connections of olfactory cortex can be divided into four classes: (1) *extrinsic*, or connections with other structures; (2) *local intrinsic* connections between different layers of POC; (3) *associative*, or connections with different parts of POC; and (4) *modulatory inputs*, or afferents that terminate in POC as part of a broader innervation of other cortical and subcortical neural systems.

Extrinsic connections: The rostral parts of POC receive terminals from both tufted and mitral cells. The caudal parts of the piriform, the periamygdaloid, and lateral entorhinal cortex receive terminals primarily or exclusively from mitral cells. In all parts of POC, the MOB projection terminates in the superficial half of layer I, designated layer Ia. There is little evidence for point-to-point topography in the projections of the MOB to POC. Anatomic studies suggest that mitral cells associated with the same glomerulus may terminate in a “patchy” or modular manner in anterior piriform cortex, with partial overlap among modules originating from different glomeruli. Cellular activity-mapping studies have provided evidence both consistent with and contrary to this idea. The major extrinsic efferent projections of POC are its reciprocal connections with the MOB and AON and its efferent projections to various nonolfactory cortical-subcortical targets (discussed later).

Local intrinsic connections: POC has two principal layers of pyramidal neurons, layers II and III, which comprise several morphologic classes and also several classes of nonpyramidal interneurons. There are extensive translaminar local connections among POC neurons. Layer II neurons give off axon collaterals to deeper layer III pyramidal cells, and there are local inhibitory interneurons in layers I and II that are contacted both by MOB terminals and by local collaterals of pyramidal cells. Deeper pyramidal cells also give rise to rich local collaterals that may synapse with local interneurons or with more superficial pyramidal cells. Thus, there are extensive translaminar connections both from superficial to deeper layers and vice versa. In addition, there are several classes of GABAergic and neuropeptide-containing local interneurons in POC.

Association connections: Cortico-cortical projections within POC are extensive and exhibit laminar and regional organization. Axons from pyramidal cells of layer II are primarily directed at more caudal sites in POC; cells in layer III project predominately to rostral parts of POC. Commissural fibers to the contralateral POC also arise from layer II of the anterior parts of POC. The ipsilateral and commissural association projections of POC terminate in a highly laminar fashion in layer Ib, immediately below the zone that contains the inputs from the MOB, with a sparse projection terminating in layer III. POC projections back to AON also terminate in layer Ib. Neurons in layers II and III send a dense projection back to the MOB; as noted previously,

this feedback pathway terminates primarily in the granule cell layer.

Modulatory inputs: POC also receives subcortical modulatory inputs from the locus coeruleus (norepinephrine), midbrain raphe nuclei (serotonergic), and magnocellular basal forebrain—the nucleus of the diagonal band—(cholinergic, and GABAergic) and from the ventral tegmental area (dopaminergic).

4.4. POC Affects a Diversity of Brain Structures

Two classes of POC outputs were discussed above: the feedback projection to the MOB and the association connections between rostral and caudal olfactory cortex. A third class of outputs is treated separately because it represents the projections of POC to brain regions not generally included in the olfactory system *per se* although their receipt of inputs from POC obviously implicates these POC targets in olfactory function. The extrinsic outputs of piriform cortex are both to cortical and subcortical structures.

Neocortical projections: The MOB projection to POC extends dorsally beyond the cytoarchitectural limits of POC into the ventral parts of the granular insular and perirhinal cortices. There are also direct projections from POC to insular and orbital cortex. Insular and orbital cortices are the primary cortical targets of ascending pathways arising in the nucleus of the solitary tract (NTS) in the medulla and appear to contain the primary cortical representations for both gustatory and visceral sensation. Thus, olfactory projections to insular and orbital cortex may be sites that integrate olfactory and gustatory signals to generate the integrated perception of flavor. These same cortical areas also have descending projections to the hypothalamus and back to the NTS; these corticofugal connections influence visceral-autonomic and possibly gustatory functions. Therefore, this circuitry could also allow olfactory modulation of autonomic function. Neurons in these cortical areas in primates respond to odors with a higher degree of selectivity than do neurons in either the MOB or POC. Thus, these neocortical sites may play a role in the discrimination of different odors.

Subcortical projections: There is a strong projection from POC to the magnocellular, medial part of the mediodorsal thalamic nucleus and the submedial nucleus (nucleus gelatinosa). These thalamic nuclei project to orbital cortex and the frontal lobes.

Direct projections to the hypothalamus arise from neurons in the deepest layers of piriform cortex and the anterior olfactory nucleus. These projections terminate most heavily in the lateral hypothalamic area. Olfactory-recipient parts of the cortical and medial amygdaloid nuclei also project to medial and anterior parts of the hypothalamus.

5. THE ACCESSORY OLFACTORY SYSTEM

5.1. *The Vomeronasal Organ and a Second, Parallel Olfactory System*

In many species, there is a second olfactory organ located at the base of the midline nasal septum in the nasal cavity, the vomeronasal organ (VNO). VNO receptor neurons (VRNs) are morphologically similar to ORNs but express two different families of odorant receptor genes that share only low homology with those present in the main olfactory epithelium. Vomeronasal receptors consist of 100 to 200 genes separated into the V1 and V2 gene families. VRNs are preferentially sensitive to low-volatility odorants including relatively large proteins. In many species, VRNs respond with high specificity and selectivity to *pheromones*, molecules emitted by conspecifics (members of the same species). Pheromones often signal the gender and/or reproductive status of the sender and can affect physiologic processes in the recipient (e.g., fertility cycles, puberty onset, etc.). VRN axons project to a specialized structure at the dorsocaudal end of the MOB called the accessory olfactory bulb (AOB). The structure and neuronal cell types of the AOB are remarkably similar to those of the MOB, but the outputs of the AOB project to brain sites that are contiguous but non-overlapping with the outputs of the MOB. This anatomic organization has given rise to the concept of two parallel olfactory systems: the main and accessory olfactory systems. Lesions of the VNO or the AOB impair reproductive behaviors and gonadosteroi d function.

The VNO is present in most human fetuses but persists to adulthood in only some individuals, and humans lack a histologically recognizable AOB. However, the central structures that receive AOB innervation in mammals with VNOs are present in humans, and these structures play similar important roles in reproductive behavior and endocrine function. Thus, it is controversial whether humans have a functional accessory olfactory system or whether odors play roles in human sexual and endocrine

functions comparable to those in mammals with accessory olfactory systems. It is possible that ORNs specialized to transduce pheromone-like odorants exist but are not anatomically segregated from ORNs in humans. To date, few VRN receptor genes have been cloned from human tissue, and at the chromosomal level, most members of this gene family are nonfunctional pseudo-genes, suggesting that their functional roles are dramatically reduced in human.

6. OLFACTION AND BEHAVIOR

Complete removal of both MOBs eliminates the ability to detect or discriminate odors. This is not particularly surprising as the bulb is the sole target of ORNs, and after *bulbectomy*, ORNs degenerate. What is somewhat surprising is that after removal of all but 10% of the MOB, experimental animals can still detect and discriminate odors, although with less precision. This suggests that odors are not represented in discrete sites in the MOB; otherwise, animals with 90% of the bulb removed should be unable to smell some odors. Contrasting with this conclusion are functional imaging experiments showing that discrete sites in the bulb have increased glomerular activity after exposure of the animal to some odorant molecules. However, other odorant molecules show a more distributed pattern of glomerular activation across broad regions of the bulb.

Most environmental odors are mixtures of many separate odorant molecules that together give the perception of the odor (e.g., coffee contains as many as 900 different volatile molecules). The question of how the olfactory system resolves these highly complex odorant mixtures is currently unknown.

Olfaction is a critical sensory system for many behaviors. For many animals, odors play an absolutely essential role in reproductive and maternal behaviors. Experimental studies have shown that bulbectomized males of some mammalian species will not mate with receptive females. In other species, olfactory cues are not absolutely essential, but mating behavior is reduced by damage to the olfactory system. In females, olfactory lesions severely impair gonadal steroid function.

In some species, odors also signal identity and social status. For example, if a pregnant female mouse is exposed to the odor of the urine of a strange male, she aborts (known as the Bruce effect), a

phenomena mediated by the accessory olfactory system. There is also evidence that the hormonal status of some animals influences their ability to detect certain odors. Female sheep, for example, become unusually sensitive to odors of their own lambs at parturition.

In humans, odors do not appear to have such profound effects on behavior and endocrine function although there have been relatively few experimental

studies of this subject. It should not be concluded, however, that olfaction is unimportant in humans. A casual glance at the ledger sheets of the food and beverage industries (remember that the perception of "flavor" is more olfaction than taste) and the magnitude of the fragrance/flavor industry leaves little doubt about the importance of olfaction and taste to the quality of our lives!

Robert W. McCarley

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1. INTRODUCTION AND ORGANIZATION OF SLEEP-WAKEFULNESS

Understanding the brain basis of consciousness is one of the oldest dreams of neuroscientists and physicians. Although knowledge of its subtleties is at a very early stage, we are now beginning to understand some of the basic mechanisms controlling the changes of consciousness associated with sleep and wakefulness.

1.1. The Electroencephalogram Is an Important, Although Limited, Tool for the Study of States of Consciousness

We begin with a brief review of the biological basis of the electroencephalogram (EEG) and evoked potentials (EPs). When activity is synchronous,

neurons in the cerebral cortex generate electrical signals strong enough to be detected through the skull by sensors (electrodes) placed on the scalp. These small (microvolt) electrical signals are amplified and filtered to produce EEG recordings. Although the EEG is a crude way to determine brain activity (similar to figuring out what is happening in a football game by putting microphones outside the stadium), it has proved to be a remarkably useful tool for studying the basic structure of sleep in humans.

Figure 1 illustrates, for one cortical neuron, the source of currents underlying the EEG. Influx of positive ions into the soma of the neuron (following a depolarizing postsynaptic potential) generates a current “sink,” as, by convention, current is composed of positive ion flow. The apical dendrites of this cortical neuron, in contrast, act as a “source” of positive ions and current flow. This current flow pattern of a source in the dendrites and a sink in the

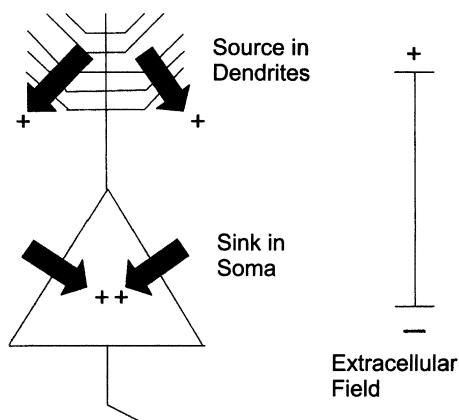


Fig. 1. Example of a cortical neuronal “dipole” occurring with a depolarizing postsynaptic potential on the soma of a pyramidal neuron. This pattern of current flow, repeated over many thousands of cortical neurons, is likely the main generator source for EEG and evoked potential waves (*see text*).

soma creates a *dipole*, literally a “two pole” with the positive pole (source) in the dendrites and the negative pole (sink) in the soma. In cerebral structures with a regular laminar structure such as the cerebral cortex, this simple dipole model repeated over many constituent neurons provides a reasonable first approximation to how positive EEG waves are generated. In the case of a hyperpolarizing postsynaptic potential in the soma, the dipole polarity would be reversed, with source (positive pole) in the soma and sink in the dendrites (it will be recalled that membrane hyperpolarization arises as the consequence of a net efflux of positive ions). Most of the components of the EEG arise from the currents generated by postsynaptic potentials, as described in our example, and not from the currents generated by action potentials. Action potentials are generally too brief and too asynchronous to summate and produce a signal detectable from the scalp. (Some of the very short latency [<10 ms] brain-stem auditory evoked potential components are derived from synchronous volleys of action potentials and are an important exception to this rule.) Evoked potentials may be thought of as EEG waves that occur after a sensory stimulus. Because they are time-locked to the stimulus, they may be averaged to improve the signal to noise ratio; this is important because the biological evoked potential signals recorded from the scalp are typically only a few microvolts in amplitude.

Many investigators are currently exploring the utility of modeling “equivalent dipoles” as a representation of the average amplitude and polarity of EEG and evoked potentials arising within a cerebral region, such as the sensory receiving areas of the

cortex. Practical constraints to localizing the source of evoked potentials and EEG include the use of scalp recordings and the consequent “smearing” of current flow as the boundaries between zones of different conductivities are traversed. For example, the brain and its extracellular fluid is a much better conductor than is the scalp. Studies have applied an experimental approach to the question of the accuracy of source localization possible with electrical signals. Using patients who had deep electrodes implanted to locate the seizure source prior to surgery, a low-level signal was passed through two deep electrodes (a true dipole source!), and then it was determined how closely the signal source within the brain could be localized from scalp electrode recordings. For this single source, brain localization was found to be accurate to within about 1 cm. However, as the number of sources increases, as is usually the case in brain processing, the ability to localize them becomes less.

The EEG is perhaps most useful in its roles in detecting the presence of seizure activity and in pinpointing changes in alertness and sleep stages, a major topic of this chapter. The EEG is described in terms of the amplitude of its waves and their frequency. As shown in Table 1, EEG frequencies are grouped into bands that range from the very low frequencies (delta, 0.5 to 4 Hz) to the very fast (beta, 14 to 32 Hz; and gamma, discussed later). As a general rule, the delta EEG frequencies are associated with states of consciousness with little complex processing, such as non-rapid eye movement (non-REM) sleep, whereas those with higher frequencies are associated with more complex processing, such as occurs in wakefulness and REM sleep (dream sleep).

The *alpha rhythm* has a frequency range of 8 to 13 Hz and is best recorded over the occipital scalp region. It occurs during wakefulness, often appearing upon eye closure and disappearing with eye opening. Depth recordings in animals indicate alpha rhythm frequencies may also be present in visual thalamus (lateral geniculate body, pulvinar), and the cortical

Table 1
EEG Frequency Bands

Name	Frequency range (Hz)
Delta	0.5–4
Theta	4–8
Alpha	8–14
Beta	14–30
Gamma (<i>see text</i>)	30–60 +

component appears to be generated in relatively small cortical areas that act as epicenters. Unfortunately, there are as yet no definitive studies of the genesis of this rhythm, although interaction of cortico-cortical and thalamocortical neurons has been postulated. Origins of the delta waves will be discussed below.

The high-voltage slow-wave activity in cortex during non-REM sleep (termed *EEG synchronization*) contrasts sharply with the low-voltage fast pattern (often termed *activated*) characteristic of both waking and REM sleep and consisting of frequencies in the beta range and higher. A term often used to describe the EEG of wakefulness and REM sleep is *desynchronized*, meaning that the slow waves of non-REM sleep are not visible.

1.1.1. GAMMA ACTIVITY

It should be noted, however, that recent work indicates high-frequency (gamma) synchronized waves may be present in waking and REM sleep, although these are of low amplitude. As the term is currently used, gamma frequencies are centered on about 40 Hz and range from about 30 to 60 Hz, and even higher. Gamma activity may index synchronous activity of cortical cell columns involved in neural processing, and recent work in cognitive neuroscience suggests fast EEG activity in the gamma band (30 to 60 Hz) increases during, and may be involved in, the formation of percepts and memory, linguistic processing, and other behavioral and perceptual functions, including associative learning. Furthermore, recent work from the author's laboratory indicates gamma activity may be deficient in schizophrenia.

1.2. Sleep Is Organized into a Definite Structure

One-third of our lives is spent in sleep. No other single behavior occupies so much of our time, yet few other behaviors have been so mysterious. We now know that there are two main states of sleep, REM sleep, typically associated with a high level of brain neuronal activity and the distinctive conscious state of dreaming, and non-REM sleep, typically associated with a low level of neuronal activity and non-visual, ruminative thinking. A typical study of sleep includes records of the EEG, of eye movements (the electro-oculogram; EOG), and of muscle tone (the electromyogram; EMG). This ensemble of records is known as a *polysomnogram* and the recording process is called *polysomnography*. These key records enable us to describe the main stages of sleep. As sleep onset approaches, the low-amplitude, fast-frequency EEG of alert wakefulness, often with alpha present (Fig. 2A), yields to stage 1 sleep, a brief transitional phase

between wakefulness and "true" sleep. This stage is often called *descending stage 1* because it is a prelude to deeper sleep stages and it is characterized by low-voltage (amplitude), relatively fast frequency EEG patterns and slow, rolling eye movements. During stage 2 sleep, there are episodic bursts of rhythmic, 14- to 16-Hz waveforms in the EEG, known as *sleep spindles*, interspersed with occasional short-duration, high-amplitude *K complexes*, so named because of their morphologic resemblance to this letter. During stage 2, the EEG slows still further. Stages 3 and 4 are defined, respectively, by lesser and greater occurrence of high-amplitude, slow (0.5 to 4 Hz) waveforms, called *delta waves*. The low-voltage fast EEG pattern of REM sleep is in marked contrast with delta sleep and resembles the non-alpha EEG pattern of active wakefulness and stage 1 descending (Fig. 1A). REM sleep is further characterized by the presence of bursts of rapid eye movements (hence the name) and by loss of muscle tone in certain major muscle groups of the limbs, trunk, and neck. Often, the non-REM sleep stages are lumped together and simply termed *non-REM sleep*. (Researchers working with animals often use the term *slow-wave sleep* for *non-REM sleep*, and this term sometimes occurs in the human literature, although, properly speaking, stages 1 and 2 do not have slow waves.) Table 2 summarizes the chief differences between waking, non-REM, and REM sleep in a polysomnographic recording.

There is a rather predictable pattern of shifting between one sleep state and another during a typical night's sleep (Fig. 2B). As the night begins, there is a stepwise descent from wakefulness to stage 1 through to stage 4 sleep, followed by a more abrupt ascent back toward stage 1. However, in place of stage 1, the first REM sleep episode usually occurs at this transition point, about 70 to 90 min after sleep onset. The first REM sleep episode in humans is short. After the first REM sleep episode, the sleep cycle repeats itself with the appearance of non-REM sleep and then, about 90 min after the start of the first REM period, another REM sleep episode occurs. This rhythmic cycling persists throughout the night. The REM sleep cycle length is 90 min in humans, and the duration of each REM sleep episode after the first is approximately 30 min. Over the course of the night, delta wave activity tends to diminish, and non-REM sleep has waves of higher frequencies and lower amplitude. As Fig. 2B makes clear, body movements during sleep tend to cluster just before and during REM sleep. In general, the ease of arousal from sleep parallels the ordering of the sleep stages, with REM and stage 1 being the easiest for arousal and stage 4 the most difficult.

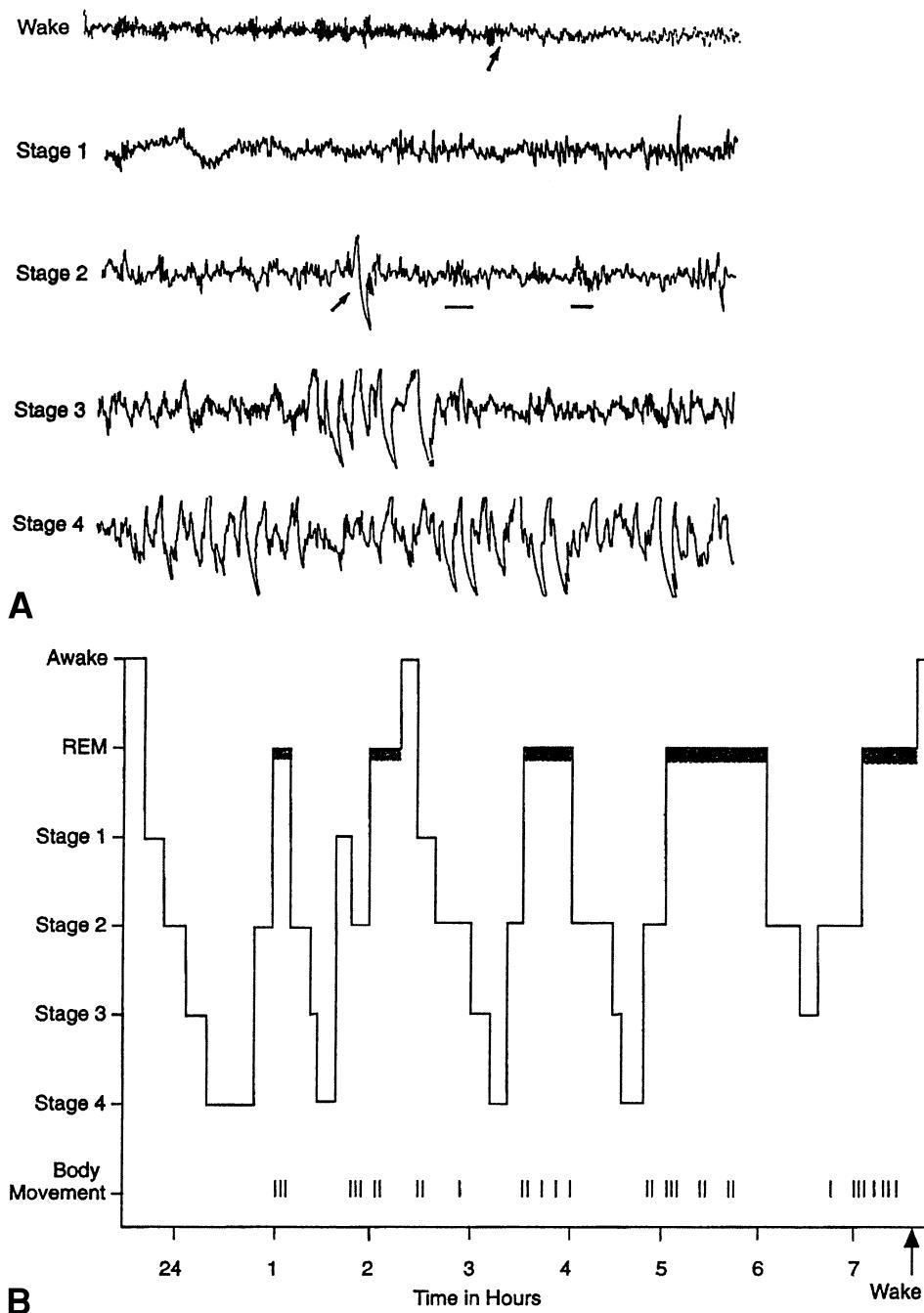


Fig. 2. (A) The EEG patterns associated with wakefulness and the stages of sleep. (B) The time course of sleep stages over a night's sleep in a healthy young man. During wakefulness, there is a low-voltage fast EEG pattern, often with alpha waves, as shown here. At the arrow there is a transition to stage 1 sleep, with loss of the alpha rhythm, and the presence of a low-voltage fast EEG. As sleep deepens, the EEG frequency slows more and more. Stage 2 is characterized by the presence of K complexes (arrow) and sleep spindles (underlined). During stage 3, delta waves (0.5 to 4 Hz) appear, and in stage 4 they are present more than 50% of the time. During REM sleep (black bars), the EEG pattern returns to a low-voltage fast pattern. The percentage of time spent in REM sleep increases with successive sleep cycles, whereas the percentage of stages 3 and 4 decreases. (EEG segments recorded from C3, except from O2 in waking, so as to show the alpha rhythm most clearly. Figure adapted from Carskadon MA, Dement WA. Normal human sleep: An overview. In: Kryger MH, Roth T, Dement WC (eds). *Principles and Practices of Sleep Medicine*. New York: Saunders, 1989, pp. 3–13.

Table 2
Polysomnographic Definition of Wakefulness, Non-REM Sleep, and REM Sleep

State	EEG amplitude and main frequencies	Rapid eye movement (EOG)	Muscle tone (EMG)
Waking	Low-voltage, fast	+	+
Non-REM sleep	High-voltage, slow	-	-
REM sleep	Low-voltage, fast	+	-

2. SLEEP HAS DISTINCTIVE ONTOGENETIC AND PHYLOGENETIC FEATURES

Periods of immobility and rest are present in many lower animals, including insects and lizards. Because of the absence of a cortical brain structure like that of humans, it is difficult to say whether the absence of slow waves in these animals means they are not having the equivalent of human non-REM sleep or whether this is present but expressed in a different form not detectable with EEG recordings. Recent work in molecular biology suggests that evaluation of changes in gene expression in activity periods versus rest periods, as well as adenosine pharmacology (*see* discussion of adenosine later), may help evaluate similarities/differences in lower and higher animals during quiescence and non-REM sleep. REM sleep is present in all mammals, save for the exception of egg-laying mammals (monotremes), such as the echidna (spiny anteater). Birds have very brief bouts of REM sleep. REM sleep cycles vary in duration according to the size of the animal, with elephants having the longest cycle and smaller animals having shorter cycles. For example, the cat has a sleep cycle of approximately 22 min, and the rat cycle is about 12 minutes.

In utero, mammals spend a large percentage of time in REM sleep, ranging from 50% to 80% of a 24-h day. Animals born with immature nervous systems have a much higher percentage of REM sleep at birth than do the adults of the same species. For example, sleep in the human newborn occupies two-thirds of the time, with REM sleep occupying one-half of the total sleep time, or about one-third of the entire 24-h period (Fig. 3A). In infants born 10 weeks prematurely, the percentage of REM sleep in the total sleep time reaches 80%. The percentage of REM sleep declines rapidly in early childhood so that by approximately age 10, the adult percentage of REM

sleep is reached, 20% to 25% of total sleep time. Obviously, the predominance of REM sleep in the young suggests an important function in promoting nervous system growth and development (*see* Section 10). Also in favor of this functional theory is the fact that the absolute amount of REM sleep is greater at birth in animals that are born immature (altricial) than those that are born more mature (precocial).

Stage 4, defined by the presence of delta EEG frequencies, is minimally present in the newborn but increases over the first years of life, reaching a maximum at about age 10 and declining thereafter (Fig. 3B). The time course of delta wave intensity over the first three decades of life is fit by a particular probability distribution (gamma distribution), and approximately the same time course obtains for synaptic density and PET measurements of metabolic rate in human frontal cortex. It has been suggested by Feinberg and co-workers that the correlated reduction in these three variables may reflect a pruning of redundant cortical synapses that is a key factor in cognitive maturation, allowing greater specialization and sustained problem solving.

A frequent question asked of physicians is how much sleep is needed. As just discussed, the answer partly depends on the age of the individual. A good general rule is that enough sleep is needed to prevent daytime drowsiness. Each individual seems have a particular “set-point” of need. In adults, the modal value of sleep need appears close to the traditional 8 h, but there is considerable individual variation. If someone functions and feels well on less sleep, there is little need for concern.

3. SLEEP ONSET AND SLEEPINESS IS DETERMINED BY CIRCADIAN TIME OF DAY AND BY PRIOR WAKEFULNESS

3.1. Circadian Factors in Sleepiness

In adult humans, the period of maximal sleepiness occurs at the time of the circadian low point of the temperature rhythm (Fig. 4). (Circadian means about a day, “circa” = “about,” and the circadian temperature rhythm of humans can be thought of as a sine wave function with a minimum that occurs between 4 and 7 AM in subjects with a normal daytime activity schedule.) It is no accident that accidents are most frequent at the time near circadian temperature minima, as this is the time of maximal sleepiness. Per vehicle mile, the risk for truck accidents is greatest at this time. The nuclear reactor incidents at both Chernobyl

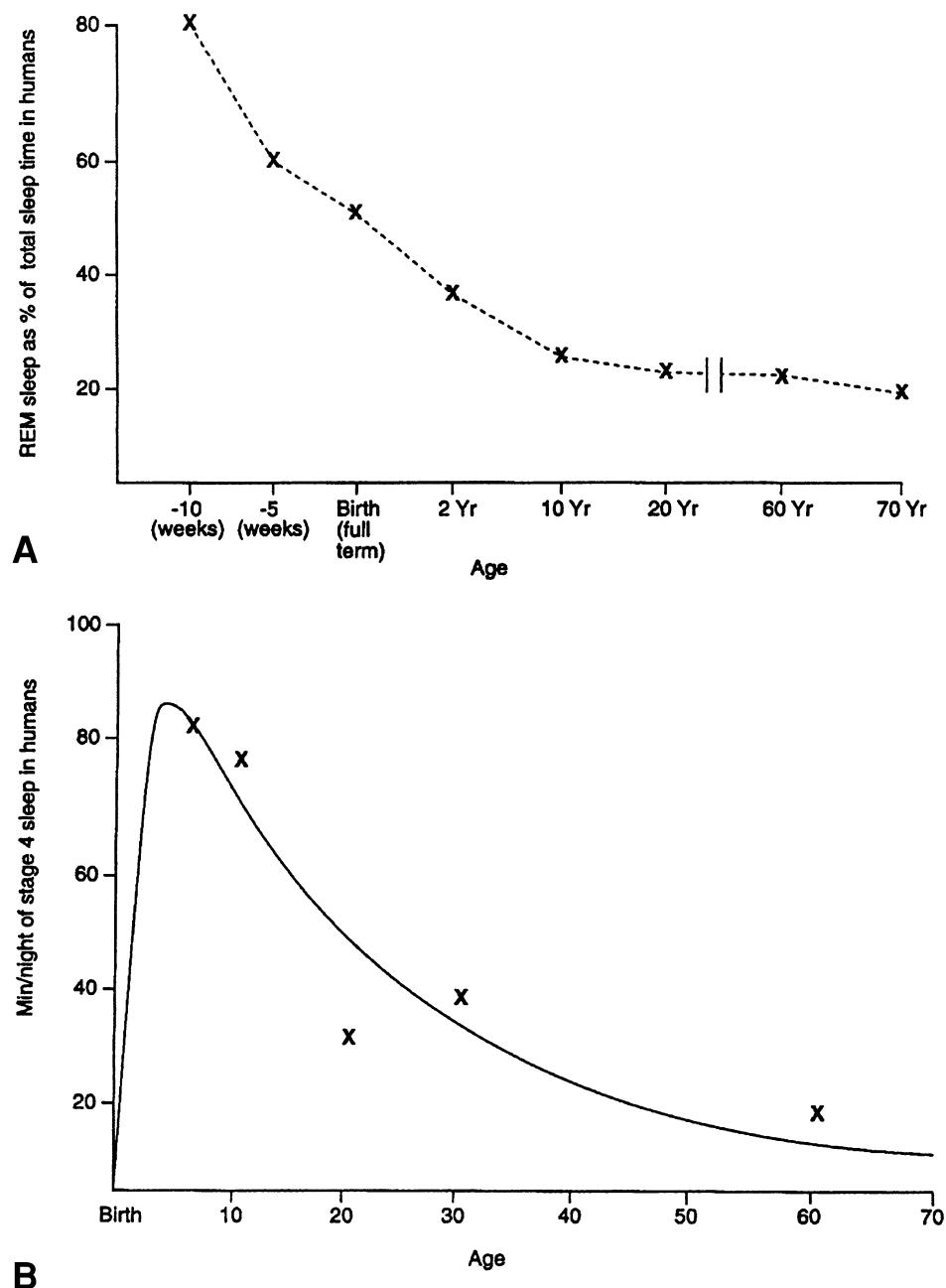


Fig. 3. (A) REM sleep as a percentage of total sleep time in infants born 10 and 5 weeks prematurely, at full-term birth, and in children and adults at the indicated years of age. Note the dramatic decline of REM sleep during early life and the long plateau during maturity, with a decline observed only in the seventh decade. (B) Stage 4 (delta) sleep minutes as a function of age. There is little delta wave activity at birth, presumably reflecting cortical immaturity. Delta wave activity peaks at about age 3 to 5 years and declines exponentially thereafter. (Figures adapted from Feinberg I, Thode HC, Chugani HT, March, JD. Gamma function describes maturational curves for delta wave amplitude, cortical metabolic rate and synaptic density J Theoret Biol 1990;142:149–161.)

and Three Mile Island also occurred in the early morning hours. There is a secondary peak of sleepiness that occurs about 3 PM (Fig. 4), corresponding with a favored time for naps. The main functional consequence of deprivation of sleep seems to be the presence of *microsleeps*, that is, very brief episodes of

sleep during which sensory input from the outside is diminished and cognitive function is markedly altered. As every parent knows, human newborns do not have a strong circadian modulation of sleep, and some species, such as the cat, do not have much circadian modulation even as adults.

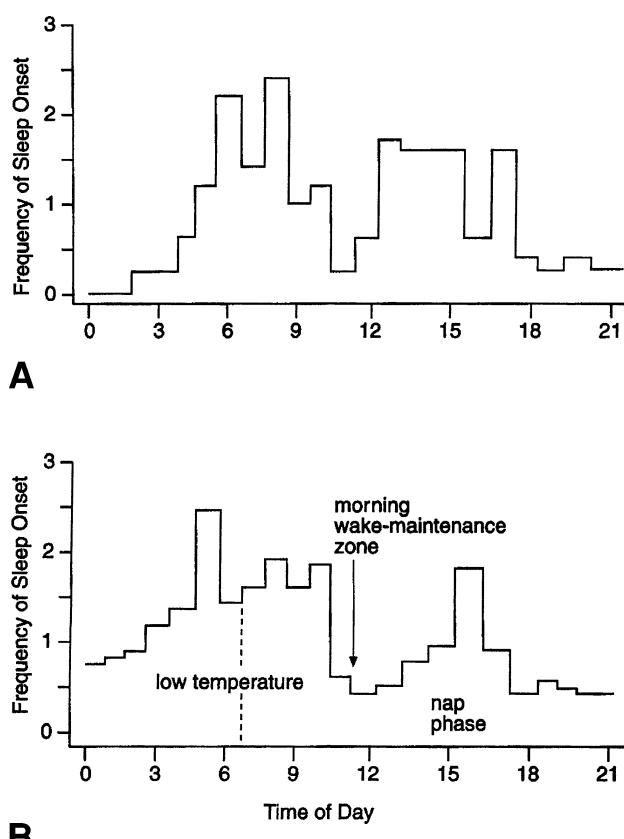


Fig. 4. Circadian control of sleepiness and sleep onset. *Top panel:* Sleepiness at various clock times for subjects on a constant routine. Sleepiness was measured by Carskadon as frequency of unintended microsleeps in subjects instructed to stay awake, with a frequency of 1 indicating the average across all measurements. Note the major peak about 6 AM, at the presumptive time of circadian temperature minimum, and a secondary peak about 3 PM, a favored time for a nap. *Bottom panel:* Sleep propensity measured as the number of self-selected bedtimes/sleep onsets in subjects in whom temperature was continuously monitored. Note that, as in the top panel, the maximum number of sleep onsets occur near temperature minimum, and a secondary peak occurs at a circadian phase corresponding with about 3 PM. These subjects were maintained without circadian cues and showed decoupling of the activity and the temperature rhythms (internal desynchronization) that are otherwise synchronized by external circadian cues, such as dawn and dusk. The sleep onsets were converted to approximate times of day by assuming a temperature minimum at 6:30 AM. (Figure adapted from Strogatz, SH. *The Mathematical Structure of the Human Sleep–Wake Cycle*. Berlin Heidelberg/New York: Springer-Verlag, 1986.)

3.2. The Second Factor Determining Sleepiness Is the Extent of Prior Wakefulness

Mathematical models of sleep propensity have been developed emphasizing circadian control and by Borbély and co-workers, who emphasize the

extent of prior wakefulness. Borbély and co-workers' model postulates that the intensity and amplitude of delta wave activity (as measured by power spectral analysis) indexes the level of sleep factor(s) and slow-wave sleep drive. In this model, the time course of delta activity over the night, a declining exponential, reflects the dissipation of the sleep factor(s). These workers did not identify the underlying sleep factor(s), but adenosine and other candidate factors are next discussed.

4. SLOW-WAVE SLEEP FACTORS

4.1. Adenosine: A Mediator of the Sleep-Inducing Effects of Prolonged Wakefulness

A growing body of evidence supports the role of purine nucleoside adenosine as a mediator of the sleepiness after prolonged wakefulness. Common-sense evidence for an adenosine role in sleepiness comes from the nearly universal use of coffee and tea to increase alertness, as these beverages contain the adenosine receptor antagonists caffeine and theophylline. The author and co-workers have advanced the hypothesis that, because of the metabolic demands during prolonged wakefulness, adenosine accumulates selectively in the basal forebrain and promotes the transition from wakefulness to slow-wave sleep (SWS) by inhibiting basal forebrain neurons important in the maintenance of wakefulness, as measured by an activated EEG.

Many studies have indicated the basal forebrain contains both cholinergic and noncholinergic neurons important in the maintenance of wakefulness (Fig. 5; cholinergic neurons use the neurotransmitter acetylcholine). There is strong evidence adenosine acts to suppress the activity of these neurons and thus promote sleep. *In vivo* work in animals demonstrated that microdialysis perfusion of adenosine in the basal forebrain zones of cholinergic neurons produced a strong reduction in wakefulness and in the activated EEG. This pointed to this region as a specifically important site of adenosine action. Key evidence that adenosine fulfilled criteria for a sleep factor mediating sleep after prolonged wakefulness was the finding that, in the basal forebrain, extracellular adenosine progressively accumulated with each succeeding hour of wakefulness (Fig. 6). Moreover, increasing basal forebrain adenosine concentrations to approximately the level as during sleep deprivation by a nucleoside transport blocker mimicked the effect of sleep deprivation on both the EEG power

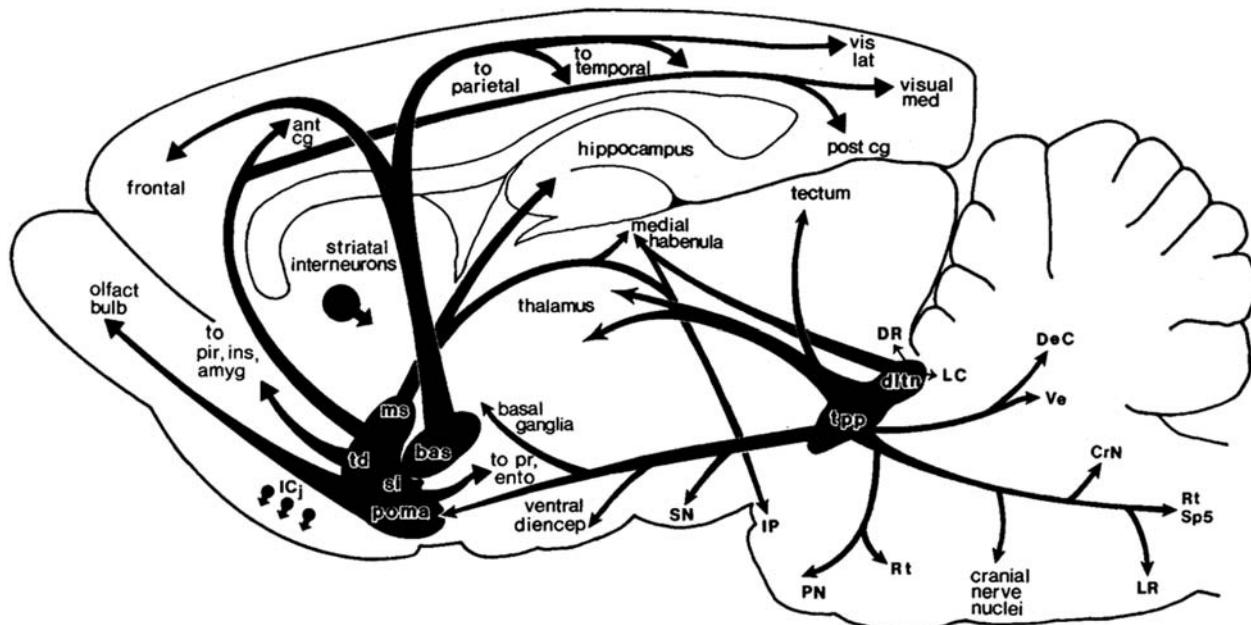


Fig. 5. Schematic of cholinergic systems and their projections in the rat. The clustered group of cholinergic nuclei in the basal forebrain form a center important for control of wakefulness and an activated EEG. These include: ms, medial septal nucleus; bas, nucleus basalis; si, substantia innominata; poma, preoptic magnocellular field; td, diagonal band nuclei. Note that projections from this group encompass almost the entire extent of the neocortex. In the brain stem, the cholinergic nuclei are tpp, pedunculopontine tegmental nucleus (abbreviated in text as PPT) and dltn, laterodorsal tegmental nucleus (abbreviated in text as LDT). The section on REM sleep discusses these nuclei in detail. (From Cooper JR, Bloom FE, Roth RH, *The Biochemical Basis of Neuropharmacology*, 7th ed. Oxford University Press, Oxford, UK, 1996.)

spectrum and behavioral state distribution: wakefulness was decreased, and there were increases in non-REM sleep. As predicted, microdialysis application of the specific A1 receptor antagonist cyclopentyltheophylline (CPT) in the basal forebrain produced the opposite effects on behavioral state, increasing wakefulness and decreasing SWS and REM. Data from combined unit recording and microdialysis studies have shown that basal forebrain neurons selectively active in wakefulness, compared with SWS, have discharge activity suppressed by both adenosine and the A1-specific agonist cyclohexyladenosine (CHA), whereas discharge activity is increased by the A1 receptor antagonist CPT.

How is the extracellular concentration of adenosine regulated? The author's current hypothesis is that regulation of extracellular concentration of adenosine depends first on metabolism, with increased metabolism leading to reduced high-energy phosphate stores and hence to increased adenosine, which, via an equilibrative transporter, leads to increases in extracellular adenosine (see schematic description in Fig. 7A). The increased extracellular adenosine then inhibits those basal forebrain neurons important in the promotion of wakefulness/cortical activation (Fig. 7B). Extracellular adenosine may

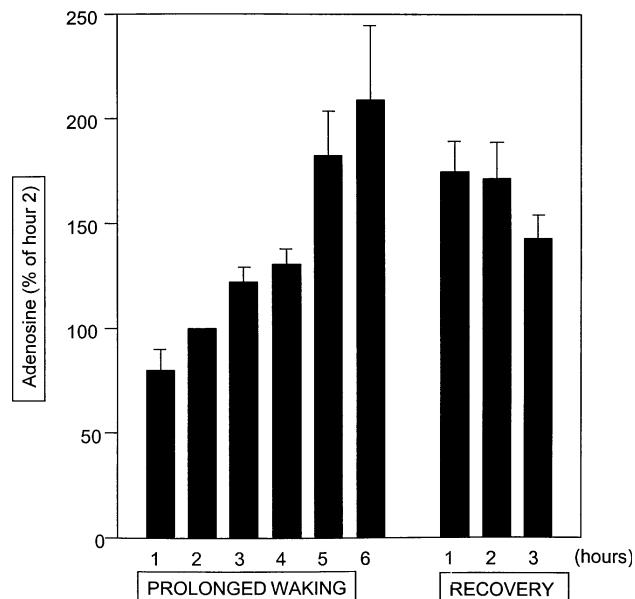


Fig. 6. Mean basal forebrain extracellular adenosine values by hour during 6 h of prolonged wakefulness and in the subsequent 3 h of spontaneous recovery sleep. Microdialysis values in the six cats are normalized relative to the second hour of deprivation. (Adapted from Porkka-Heiskanen T, Strecker RE, Thakkar M, Björkum AA, Greene RW, McCarley RW. Adenosine: A mediator of the sleep-inducing effects of prolonged wakefulness. *Science* 1997; 276:1265–1268.)

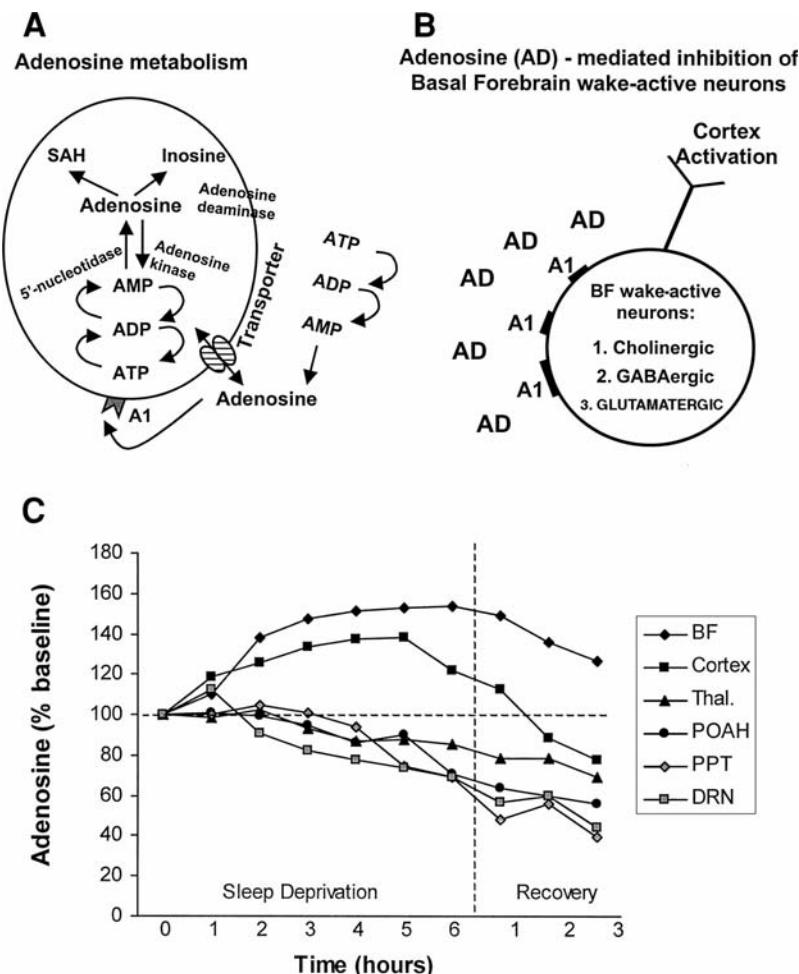


Fig. 7. (A) Schematic of main intra- and extracellular metabolic pathways of adenosine. The intracellular pathway from ATP (adenosine 5'-triphosphate) to ADP (adenosine diphosphate) to AMP (adenosine monophosphate) to adenosine is respectively regulated by the enzymes ATPase, ADPase, and 5'-nucleotidase and extracellularly by the respective ecto-enzymes. Adenosine kinase converts adenosine to AMP, and adenosine deaminase converts adenosine to inosine. The third enzyme to metabolize adenosine is *S*-adenosylhomocysteine hydrolase, which converts adenosine to *S*-adenosylhomocysteine (SAH). Adenosine concentration between the intra- and extracellular spaces is equilibrated by nucleoside transporters. (B) Schematic of the effects of adenosine on cells in the basal forebrain. Extracellular adenosine (AD) acts on the A1 adenosine receptor subtype to inhibit neurons promoting EEG activation and wakefulness. (C) Adenosine concentrations in different brain areas during sleep deprivation and recovery sleep. Cats were kept awake for 6 h using gentle handling/playing (hours 1 to 6) and then allowed to sleep for 3 h (hours 7 to 9). Prior to the beginning of the sleep deprivation, samples were collected to obtain baseline wakefulness values for each probe (predeprivation value = hour 0 = 100%). Two patterns are evident in the sleep deprivation-induced changes in adenosine; in the basal forebrain (BF) and cortex, adenosine levels could be seen to rise during the sleep deprivation, whereas in the other four areas, adenosine levels were either stable or declined slowly during the 6 h of sleep deprivation. By the second hour of sleep deprivation, adenosine values were significantly higher in the BF than in all other brain areas but cortex (Ctx), which was lower in hour 6. During recovery, sleep adenosine concentrations were significantly higher in the BF than in all other areas. DRN, dorsal raphe nucleus; POA, preoptic hypothalamic area; PPT, pedunculopontine tegmental area.

also be increased by the release of adenosine triphosphate (ATP) as a co-transmitter and its breakdown, by 5'ectonucleotidases, to adenosine. Support for a adenosine-metabolism link hypothesis comes from the fact that EEG arousal is known to diminish as a function of the duration of prior wakefulness and also with brain hyperthermia, both associated with

increased brain metabolism, as well as data indicating glycogen depletion during wakefulness and restoration during non-REM sleep. The release of nitric oxide (NO) also has been strongly implicated in the increased adenosine levels with sleep deprivation, both by *in vivo* and *in vitro* work. The mechanism by which NO increases adenosine is under investigation.

In terms of adenosine effects, not only is there neuronal activity suppression from increased extracellular adenosine, but also prolonged periods of exposure to increased levels of adenosine produce a positive feedback on the number of active receptors for adenosine (the A1 receptor), increasing their number. This increase in the number of active receptors is a second mechanism of homeostatic feedback that occurs with longer periods of wakefulness and further promotes sleep.

Does adenosine exerts its effects in localized brain region(s) or globally? The initial brain region in which extracellular adenosine increases with wakefulness is the basal forebrain. Measurements in multiple brain areas in the cat showed sustained adenosine accumulation during periods of wakefulness up to 6 h occurred only in the basal forebrain and to a lesser extent in cerebral cortex. Adenosine concentrations did not increase elsewhere during wakefulness, even in brain regions important in behavioral state control (Fig. 7C). These data suggest the presence of brain region-specific differences in adenosine transporters and/or degradation. At longer time intervals of wakefulness from sleep deprivation, 12 to 24 h in humans, many regions of cerebral cortex show evidence of increased adenosine, as measured by PET detection of the presence of increased numbers of adenosine A1 receptors.

4.1.1. ADENOSINE AND SLEEP DEBT

With continuing sleep restriction, such as 5 h per night in humans, there are cumulative and long-term changes in alertness and cognitive performance that occur, which are often termed *sleep debt*. Recent data from the author's laboratory indicate that sleep deprivation may alter transcriptional activity in the basal forebrain via an adenosine-induced induction of the transcriptional factor, nuclear factor kappa B (NF- κ B), which is known to bind to the promotor region of the adenosine A1 receptor. Data suggest this adenosine- and sleep deprivation-induced NF- κ B may lead to the increased expression of adenosine A1 receptors as found to be present in the binding studies in animals and humans. This increased number of receptors will increase the sensitivity to adenosine and provide a possible mechanism for the production of persisting sleep debt. The time course of reduction in the number of adenosine A1 receptors with sufficient recovery sleep remains to be determined, although the time course of sleep debt reduction suggests this should be of the order a weekend, or 2 days.

4.1.2. ACTIVE NON-REM SLEEP-PROMOTING MECHANISMS. THE VENTROLATERAL PREOPTIC REGION

Electrophysiologic recordings of basal forebrain/anterior hypothalamic neurons indicate that some are selectively active during non-REM sleep and might represent an active sleep-promoting mechanism. Most importantly, work by Sherin and co-workers used the immediate early gene protein product c-Fos to detect neurons in the ventrolateral preoptic area (VLPO) that were selectively active during non-REM sleep; immunohistochemistry indicated these neurons were GABAergic, and anatomic studies indicated projections to wakefulness-promoting histaminergic neurons in posterior hypothalamus and to brain-stem nuclei important in EEG arousal. The interaction of these neurons with other systems important in sleep is being determined. One conceptualization is that the adenosine released in the basal forebrain suppresses activity in wake-active neurons, and this permits activity in VLPO neurons that "latches" the brain in a relatively persisting non-REM state.

4.1.3. OTHER HUMORAL FACTORS

There is also evidence supporting a role for interleukin-1, tumor necrosis factor, and growth hormone releasing hormone as part of the humoral mechanisms regulating physiologic sleep. Their injection enhances non-REM sleep, whereas their inhibition reduces spontaneous sleep and sleep rebound after sleep deprivation. Changes in their mRNA levels and changes in their protein levels in the brain are consistent within their proposed role in sleep regulation, as are results from transgenic and mutant animals. However, they appear to be involved in regulation of propensity to sleep over longer time periods than the actions of adenosine. Of note, interleukin-1 is a cytokine that is produced in response to infections and, with infections, increases non-REM sleep and also produces hyperthermia. Hyperthermia itself may increase non-REM sleep, but blocking the hyperthermic effects of interleukin-1 does not block the non-REM sleep-inducing effects. The argument that interleukin-1 is important in the hypersomnia associated with infections is thus strong, and interleukin-1 may also play a role in producing sleep after sleep deprivation.

Hayaishi and co-workers have reported that injections of prostaglandin D2 into the third ventricle and the ependymal surface of the ventral forebrain reliably produces slow-wave sleep. They have proposed that it is a natural sleep regulatory factor. Interestingly, Hayaishi and co-workers have found

that at least some of the sleep-inducing effects of prostaglandin could be mediated by changes in extracellular adenosine, and Krueger and co-workers have suggested a model in which some of the effects of interleukin-1 might be mediated by adenosine. The possibility exists, then, that adenosine might be a “final common factor” for some other sleep factors.

5. CONTROL OF EEG SYNCHRONIZATION AND DESYNCHRONIZATION

The high-voltage slow-wave activity in cortex during most non-REM sleep (termed *EEG synchronization*) contrasts sharply with the low-voltage fast pattern (termed *desynchronized* or *activated*) characteristic of both waking and REM sleep and consisting of frequencies in the beta range (approximately 14 to 32 Hz). We have already discussed the basal forebrain component of this activating system. As shown in Fig. 5, there are also important brain-stem components of the cholinergic activating system. This is likely a major component of the *ascending reticular activating system*, a concept that arose from the work of Moruzzi and Magoun before methods were available for labeling of neurons using specific neurotransmitters. The brain-stem cholinergic nuclei at the pons-midbrain junction are termed the *laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT)*. There is also extensive anatomic evidence that these cholinergic neurons project to thalamic nuclei important in EEG desynchronization and synchronization. Both *in vivo* and *in vitro* neurophysiologic studies have indicated that the target neurons in the thalamus respond to cholinergic agonists in a way consistent with EEG activation, as detailed later. Cholinergic systems are not the exclusive brain-stem substrate of EEG desynchronization. Brain-stem reticular neuronal projections to thalamus, likely using excitatory amino acid (EAA) neurotransmission, and noradrenergic projections from locus coeruleus and serotonergic projections from the dorsal raphe nucleus also play important roles in EEG desynchronization. Wakefulness appears to be too important to be left to maintenance by one neurotransmitter system.

5.1. Sleep Spindles

Spindles occur during stage 2 of human sleep and in the light slow-wave sleep phase of animals. They are composed of waves of approximately 10 to 12 Hz

frequency; the wave amplitude waxes and then wanes over the spindle duration of 1 to 2 s. Wave frequency varies between species and is higher in primates. Spindles are relatively well-understood at the cellular level. Studies by Steriade and co-workers indicate spindle waves arise as the result of interactions between spindle pacemaker GABAergic thalamic nucleus reticularis (RE) neurons and thalamocortical neurons. Spindle waves are blocked by cholinergic brain stem-thalamus projections, which act to hyperpolarize the RE neurons. The forebrain nucleus basalis also provides cholinergic and hyperpolarizing GABAergic input to RE that assists brain-stem input in disrupting the spindles.

5.2. Delta EEG Activity

The cellular basis of delta waves (0.5 to 4 Hz) originating in thalamocortical neurons is sketched in Fig. 8. This sketch portrays intracellularly recorded events in a thalamocortical neuron during delta wave generation and is based on both *in vivo* and *in vitro* recordings by McCormick and by Steriade and their co-workers. The basic concept is that a hyperpolarized membrane potential permits the occurrence of delta waves in thalamocortical circuits. Any factors depolarizing the membrane will block delta waves. During waking, input from the cholinergic forebrain nucleus basalis is important for suppression of slow-wave activity, as shown by lesion studies. Also, brain-stem norepinephrine and serotonergic projections may disrupt delta activity in waking, although they are inactive during REM sleep. During REM sleep, cholinergic input from brain stem is a major factor producing membrane depolarization, with reticular formation input, likely using EAA neurotransmission, also playing an important role. This membrane depolarization leads to suppression of delta wave activity. Thus, delta waves during sleep may be seen to represent thalamocortical oscillations occurring in the absence of activating inputs. From the standpoint of the cellular physiologist, the relative intensity of cortical desynchronization correlates well with the intensity of cholinergic input to thalamus; conversely, the relative intensity of cortical synchronization, including delta waves, correlates well with the relative absence of cholinergic activity. The identification of desynchronizing processes in sleep with ascending brain-stem cholinergic and reticular activation means that the increasing intensity of EEG desynchronization preceding REM sleep is related to the increasing level of activity of REM-related

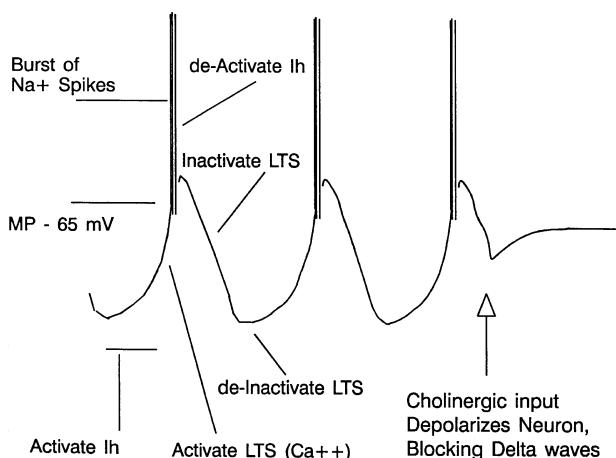


Fig. 8. Schematic of mechanisms proposed for generation of delta waves by thalamocortical neurons, which, without exogenous input, show a spontaneous membrane potential and action potential oscillation in the delta frequency and, it is hypothesized, drive the cortical delta rhythm. Oscillation occurs because of the following interplay of intrinsic membrane currents: When the membrane potential is hyperpolarized (-80 mV), a particular cation current, called I_h (I = symbol for current, h = hyperpolarized), is activated. This inward flow of positive ions depolarizes the membrane to the point where the low-threshold spike (LTS) is activated. The in-rush of calcium ions in the LTS further depolarizes the neuron to the point where the firing threshold of the sodium action potential is crossed, and action potentials are produced. I_h is turned off or “deactivated” at depolarized potentials. The LTS current is automatically turned off by another process called “inactivation.” The membrane then returns to its baseline, hyperpolarized level where the LTS calcium current is “de-inactivated” or rendered ready for activation. The cycle then repeats itself. Delta oscillations are halted by exogenous, depolarizing input, such as the cholinergic input illustrated, as reviewed in Steriade M and McCarley RW, Brain control of wakefulness and sleep. New York: Kluwer Academic/Plenum, New York, 2005.

cholinergic and reticular activity that precedes this state (see discussion in next section.)

5.3. Slow-Wave Sleep at the Cellular Level in the Thalamus: The “Burst Mode” of Relay Cell Discharge Is Responsible for the Failure of Information Transmission

Extracellular recordings by the author and Benoit demonstrated that dorsal lateral geniculate relay neurons discharged in stereotyped bursts during non-REM sleep but not during waking or REM. Subsequent *in vivo* (by Steriade and co-workers) and *in vitro* investigations (by McCormick and co-workers) indicate the bursting in thalamocortical neurons occurs when the membrane is hyperpolarized, as

illustrated in Fig. 8 in association with the delta EEG rhythm. This hyperpolarization removes the inactivation of particular Ca^{2+} channels and enables the production of a *calcium spike* (e.g., an inrush of depolarizing calcium ions) when a small depolarization occurs. This depolarizing calcium spike is termed a *low threshold spike* (LTS) to distinguish it from other calcium currents with different triggering thresholds. The LTS depolarizes the neuron sufficiently to reach the threshold for fast sodium action potentials, and a burst of these action potentials rides on the LTS. However, the production of a LTS limits the following frequency of relay neurons and hence blocks rapid information transmission.

6. REM SLEEP PHYSIOLOGY AND RELEVANT BRAIN ANATOMY

6.1. Overview and Summary: REM-On Neurons

It should be emphasized that there are two distinctly different kinds of discharge patterns in brain-stem cholinergic neurons. One type has maximal discharge rate in activated EEG states of both REM and non-REM sleep and has been discussed extensively in conjunction with non-REM sleep. However, another type has maximal activity during REM sleep but is relatively silent during both wakefulness and non-REM sleep. This type of cholinergic neuron, termed a *REM-on* neuron, is thought to be important in the generation of the state of REM sleep. Figure 9 schematizes the time course of discharge activity of the REM-on neurons. Activity in this group of neurons recruits activity in effector neurons located in the brain-stem reticular formation to produce REM sleep phenomena. Neurons in the locus coeruleus using norepinephrine and neurons in the dorsal raphe using serotonin have an opposite time course, becoming selectively inactive during REM, and are termed *REM-off* neurons (Fig. 10 sketches the location of these nuclei). They act to suppress REM sleep-promoting activity, as discussed in detail later. The legend of Fig. 9 describes the REM-on and REM-off dynamics that produce the REM cycling evident in the figure.

6.2. Transection Studies Show That the Brain Stem Contains the Neural Machinery of the REM Sleep Rhythm

As illustrated in Fig. 10, a transection made just above the junction of the pons and midbrain produces a state in which periodic occurrence of REM

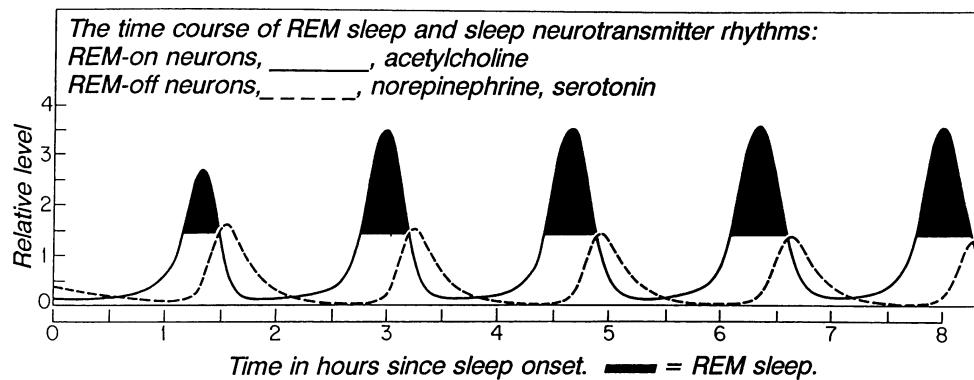


Fig. 9. Schematic of a night's course of REM sleep in humans showing the occurrence and intensity of REM sleep as dependent upon the activity of populations of REM-on (REM-promoting neurons), indicated by the solid line. As the REM-promoting neuronal activity reaches a certain threshold, the full set of REM signs occurs (black areas under curve indicate REM sleep). Note, however, that unlike the step-like EEG measure of stage in Fig. 2, the underlying neuronal activity is a continuous function. The neurotransmitter acetylcholine is thought to be important in REM sleep production, acting to excite populations of brain-stem reticular formation neurons to produce the set of REM signs. Other neuronal populations using the monoamine neurotransmitters serotonin and norepinephrine are likely REM-suppressive; the time course of their activity is sketched by the dotted line. (These curves mimic actual time courses of neuronal activity, as recorded in animals, and were generated by a mathematical model of REM sleep, the limit cycle reciprocal interaction model of McCarley and Massagoue, an elaboration of the simple Lotka-Volterra model. Lotka and Volterra modeled the waxing and waning of two populations of animals; the prey population, analogous to the REM-on neuronal population, would increase until the increased availability of food would cause the predator population, analogous to the REM-off population, to increase, until sufficient prey were eaten to decrease the food supply of the predator population, which would then decrease.)

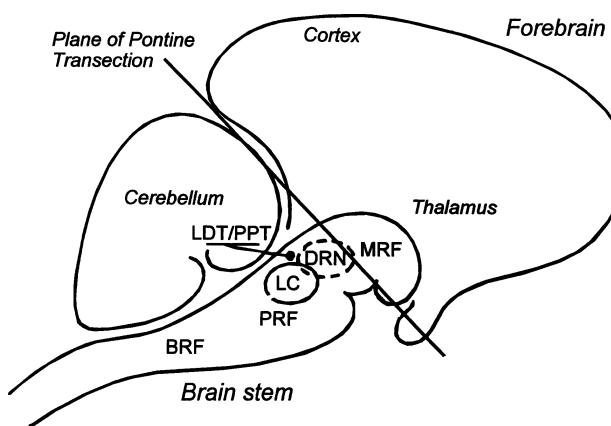


Fig. 10. Schematic of a sagittal section of a mammalian brain (cat) showing the location of nuclei especially important for sleep. BRF, PRF, and MRF, bulbar, pontine, and mesencephalic reticular formation; LDT/PPT, laterodorsal and pedunculopontine tegmental nuclei, the principal site of cholinergic (acetylcholine-containing) neurons important for REM sleep and EEG desynchronization. LC, locus coeruleus, where most norepinephrine-containing neurons are located; RN, dorsal raphe nucleus, the site of many serotonin-containing neurons; HYP, hypothalamus. The oblique line is the plane of transection that preserves REM sleep signs caudal to the transection but abolishes them rostral to the transection. (Adapted from McCarley, RW. The biology of dreaming sleep. In: Kryger MH, Roth T, Dement WC (eds). Principles and Practice of Sleep Medicine. New York: Saunders, 1989, pp. 173–183.)

sleep can be found in recordings made in the isolated brain stem, whereas recordings in the isolated forebrain show no sign of REM sleep. These lesion studies by Jouvet and co-workers in France established the importance of the brain stem in REM sleep.

6.3. Brain-Stem Reticular Formation Neurons Are Important as Effectors in the Production of the Physiologic Events of REM Sleep

The cardinal signs of REM sleep in lower animals, as in humans, are muscle atonia, EEG desynchronization (low-voltage fast-pattern), and rapid eye movements. EEG depth recordings in animals show another important component of REM sleep, the *PGO waves*, so termed because they are recorded from the pons, the lateral geniculate nucleus, and the *occipital cortex*. They are visible in the recording from the cat LGN in Fig. 11. (The depth recordings necessary to establish their presence in humans have not been done.) PGO waves arise in the pons and are then transmitted to the thalamic lateral geniculate nucleus and to the visual occipital cortex. PGO waves represent an important mode of brain-stem activation of the forebrain during REM sleep and are also present in nonvisual thalamic nuclei.

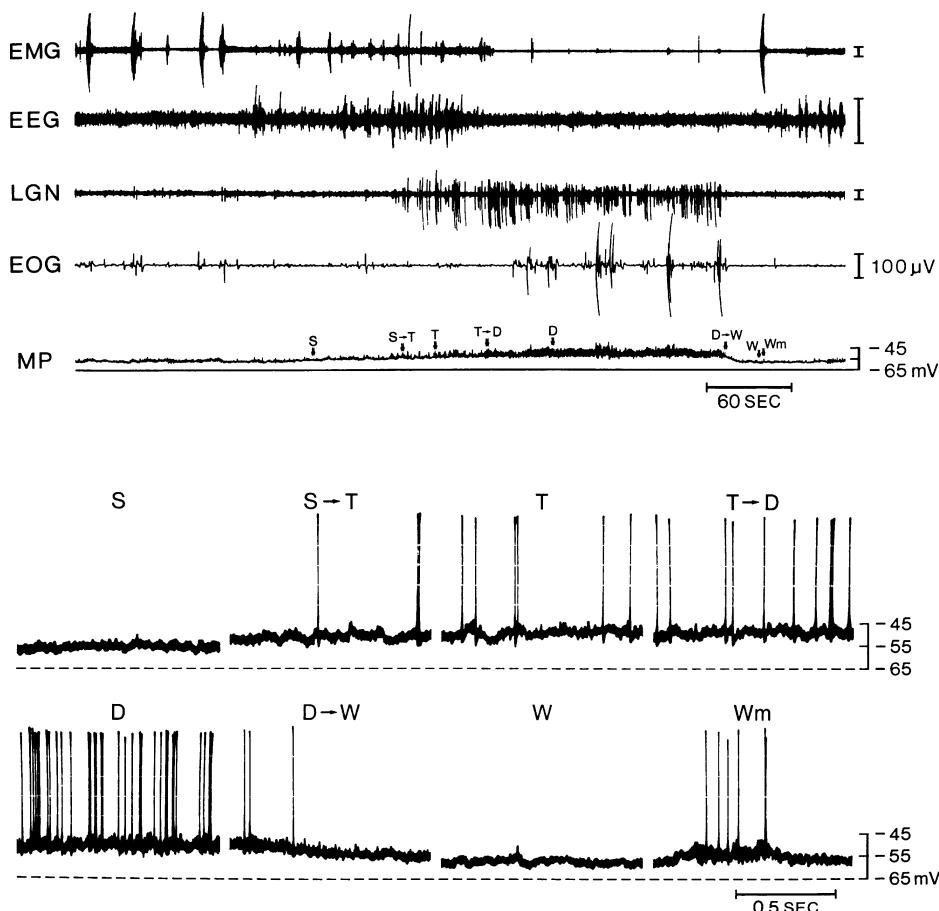


Fig. 11. REM sleep in the EEG and at the brain-stem cellular level. *Top panel:* Continuous polysomnographic record of waking, non-REM sleep, REM sleep, and return to waking in the cat. Waking is indicated by EMG activity, low-voltage fast EEG, eye movements (EOG). Non-REM sleep (here abbreviated S, for slow-wave sleep) shows high-voltage slow waves in the EEG; the transition to REM sleep (REM sleep is here abbreviated by D, for desynchronized sleep) is heralded by the onset of spiky waves in the lateral geniculate nucleus EEG recording (PGO waves), and with the occurrence of REM sleep there is muscle atonia, low-voltage fast EEG, PGO waves, and rapid eye movements. The *bottom trace* is of the membrane potential (MP) of an intracellularly recorded pontine reticular formation neuron with the action potentials filtered out; note the membrane potential depolarization that begins before and remains present throughout REM sleep. The *bottom panel* shows samples of the oscilloscope record of the intracellular recording and the occurrence of action potentials and postsynaptic potentials at the times indicated on the MP tracing. S = slow-wave sleep = non-REM sleep; T, transition; D, REM sleep; W, waking. S→T, beginning of transition to REM sleep, as indicated by PGO waves; T→D, onset of REM sleep; D→W, transition to waking; Wm, waking with body movement and action potentials in the neuron. (Data from Ito K, Yanagihara M, Imon H, Dauphin L, McCarley RW. Intracellular recordings of pontine medial gigantocellular tegmental field neurons in the naturally sleeping cat: Behavioral state-related activity and soma size difference in order of recruitment. *Neuroscience* 2002; 114:23–37.)

Most of the physiologic events of REM sleep have effector neurons located in the brain-stem reticular formation, with many important neurons concentrated in the pontine reticular formation (PRF). Thus PRF neuronal recordings are of special interest for information on mechanisms of production of these events. Intracellular recordings of pontine reticular formation neurons (Fig. 11) show that these neurons have relatively hyperpolarized membrane

potentials and generate almost no action potentials during non-REM sleep. As illustrated in Fig. 11, PRF neurons begin to depolarize even before the occurrence of the first EEG sign of the approach of REM sleep, the PGO waves that begin 30 to 60 s before the onset of the rest of the polysomnographic signs of REM sleep. As PRF neuronal depolarization proceeds and the threshold for action potential production is reached, these neurons begin to discharge

(generate action potentials). Their discharge rate increases as REM sleep is approached and the high level of discharge is maintained throughout REM sleep, due to the maintenance of a membrane depolarization.

Throughout the entire REM sleep episode, almost the entire population of PRF neurons remains depolarized. The resultant increased action potential activity leads to the production of those REM sleep physiologic signs that have their physiologic bases in pontine reticular formation neurons. Figure 12 provides a schematic overview of REM sleep as arising from increases in excitability and discharge activity of the various populations of reticular formation neurons that are important as effectors of REM sleep phenomena. Pontine reticular formation neurons are important (1) for the rapid eye movements (the generator for saccades is in PRF); (2) for the PGO waves (a different group of neurons); and (3) because a group of dorsolateral PRF neurons controls the muscle atonia of REM sleep (these neurons become active just before the onset of muscle atonia). Neurons in midbrain reticular formation (MRF; *see* anatomic schematic in Fig. 9) are especially important for EEG desynchronization, for the low-voltage fast

EEG pattern. As mentioned earlier, these neurons were originally described by Moruzzi and Magoun as making up the *ascending reticular activating system* (ARAS), the set of brain-stem neurons important for EEG desynchronization. Subsequent work has enlarged this original ARAS concept to include cholinergic and monoaminergic neurons. Neurons in the bulbar reticular formation are important for muscle atonia.

6.4. Cholinergic Mechanisms Are Important for REM Sleep

Extensive work has led to an appreciation of the importance of the neurotransmitter acetylcholine for REM sleep and to a reasonably detailed knowledge of the nature of the anatomy and physiology of the cholinergic influences on REM sleep. The essential points are outlined below.

1. Injection of compounds that are acetylcholine agonists into the pontine reticular formation produces a REM-like state that very closely mimics natural REM sleep. The latency to onset and duration are dose dependent. Muscarinic receptors

REM Sleep State Control Schematic

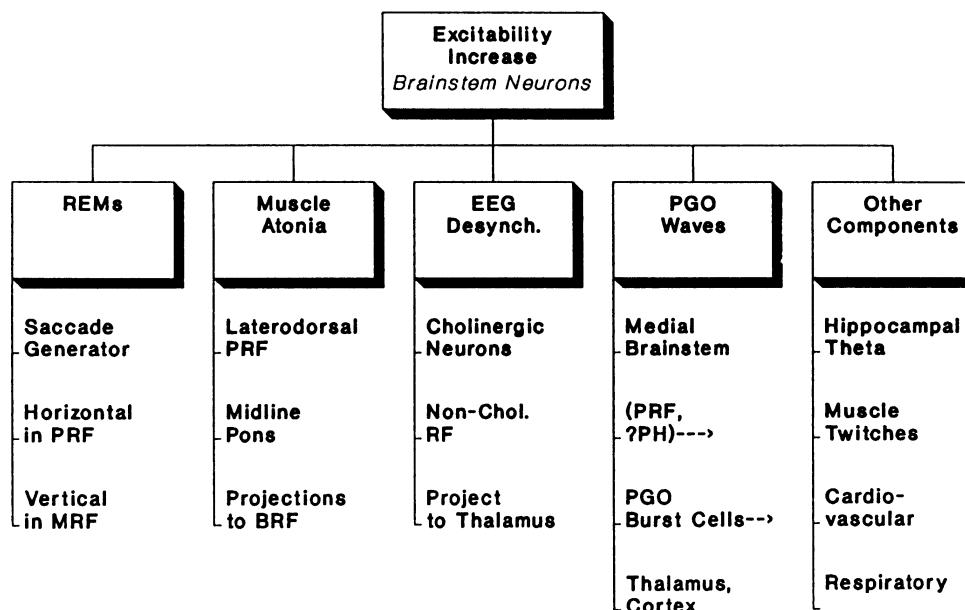


Fig. 12. Schematic of REM sleep control. Increasing the excitability (activity) of brain-stem neuronal pools subserving each of the major components of the state causes the occurrence of this component. For example, the neuronal pool important for the REMs (the rapid eye movements) is suggested to be the brain-stem saccade generating system whose main machinery is in paramedian pontine reticular formation. Although vertical saccades are fewer in REM, their presence suggests similar involvement of the mesencephalic reticular formation. Information under the other system components sketches the major features of the anatomy and projections of neuronal pools important for muscle atonia, EEG desynchronization, and PGO waves, and the last part of the diagram lists other components of REM sleep.

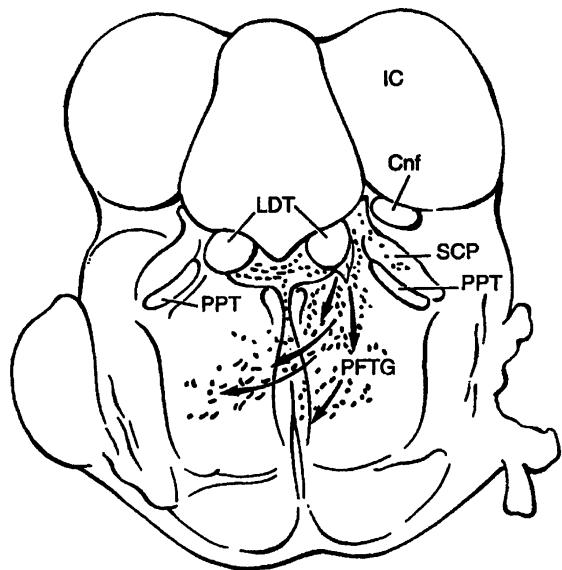


Fig. 13. Coronal section of the brain stem at the pons-midbrain junction showing the location of the acetylcholine-containing neurons most important for REM sleep in LDT/PPT (laterodorsal tegmental nucleus/pedunculopontine tegmental nucleus), and a schematic of projections of LDT to pontine reticular formation. (PFTG is an abbreviation of one component of PRF). IC, inferior colliculus; Cnf, cuneiform nucleus; scp, superior cerebellar peduncle. (Figure adapted from Mitani A, Ito K, Hallanger AE, Wainer BH, Kataoka K, McCarley RW. Cholinergic projections from the laterodorsal and pedunculopontine tegmental nuclei to the pontine gigantocellular tegmental field in the cat. *Brain Res.* 1988; 451:397–402.)

appear to be especially critical, with nicotinic receptors of lesser importance.

2. There are naturally occurring cholinergic projections to effector neurons in brain-stem reticular formation. These arise in the two nuclei at the pons-midbrain junction (Fig. 13): the *laterodorsal tegmental nucleus (LDT)* and the *pedunculopontine tegmental nucleus (PPT)*.
3. *In vitro* studies in the pontine reticular formation slice preparation show that a majority (80%) of reticular formation neurons are excited by cholinergic agonists, with muscarinic effects being especially pronounced. *In vitro* studies in the pontine reticular formation slice preparation show that the increased excitability and membrane depolarization produced by cholinergic agonists is a direct effect, as it persists when synaptic input has been abolished by addition of tetrodotoxin, which blocks sodium-dependent action potentials.
4. Experiments lesioning the LDT/PPT nuclei confirm their importance in producing REM sleep phenomena. Destruction of the cell bodies of LDT/PPT

neurons by local injections of excitatory amino acids leads to a marked reduction of REM sleep.

5. Work by McCarley, Sakai, Steriade and their co-workers have shown that a group of LDT/PPT neurons discharges selectively in REM sleep and the onset of activity begins before the onset of REM sleep. This LDT/PPT discharge pattern and the presence of excitatory projections to the PRF suggest that these cholinergic neurons may be important in producing the depolarization of reticular effector neurons for REM sleep events. The group of LDT/PPT and reticular formation neurons that become active in REM sleep are often referred to as REM-on neurons.
6. Cholinergic neurons are important in production of the low-voltage fast (LVF) EEG pattern (representing *cortical activation*) in both REM sleep and in waking. As shown by Steriade and co-workers, a different group of cholinergic neurons in LDT/PPT is active during this LVF pattern in both REM and waking. As described, this cholinergic system is especially important in generating the LVF EEG pattern, often called the *activated EEG*. Also playing a role in forebrain activation are projections from midbrain reticular neurons and aminergic neurons, especially those in locus coeruleus. Together these neuronal groups form the ARAS. Evidence that multiple systems are involved in EEG desynchronization comes from the inability of lesions of any single one of these systems to disrupt EEG desynchronization on a permanent basis.

Figure 14 shows a structural model of REM sleep control. (The reader is cautioned that our understanding of REM sleep control is an evolving process and that this schematic represents the author's interpretation of current evidence.) The REM-on neurons in this figure include the cholinergic neurons in LDT/PPT (labeled no. 1) whose actions in REM generation have been discussed. During REM sleep, cholinergic neurons in the LDT/PPT have two major actions on reticular formation neurons. Most reticular formation neurons are excited by REM-on cholinergic input and are consequently REM-on themselves. These constitute the reticular formation effector neurons (no. 2A in the figure), which can be thought of as premotor neurons for the characteristic events of REM sleep, as has been described above. This REM-on group of reticular formation neurons has positive excitatory feedback actions on the LDT/PPT cholinergic neurons (Fig. 14). A second action of REM-on cholinergic input is to inhibit another, smaller group of reticular neurons, which

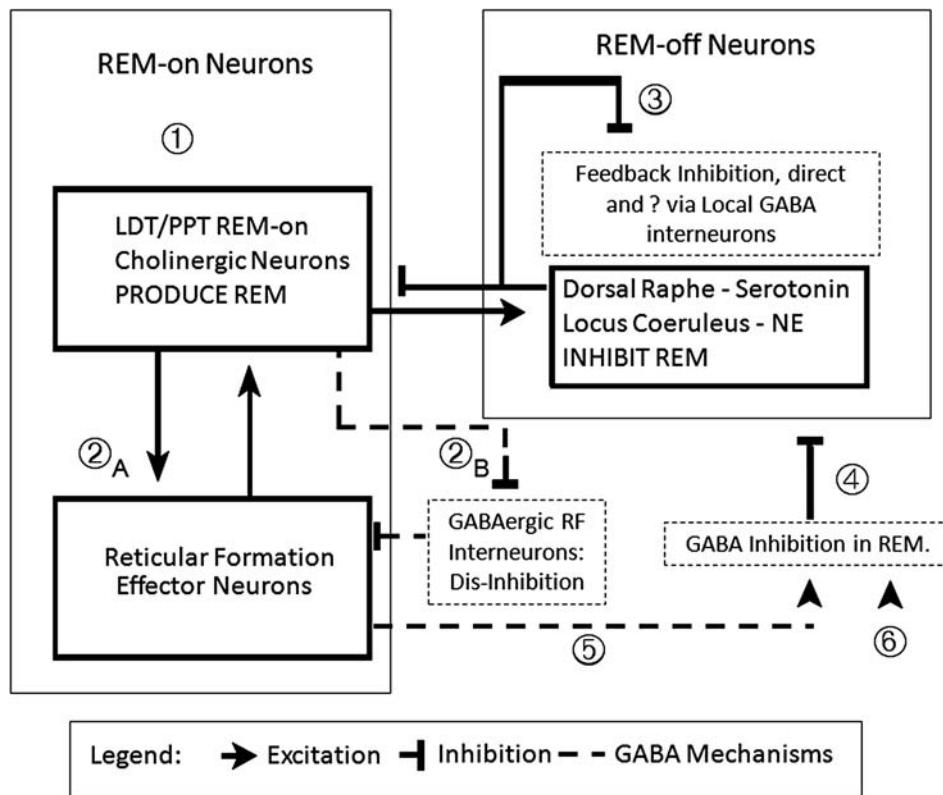


Fig. 14. Structural model of REM sleep control (see text for discussion).

are likely GABAergic reticular formation neurons (no. 2_B in the figure) with inhibitory projections to reticular formation REM-on neurons. Cholinergic REM-on inhibition of GABAergic neurons will thus lead to, by virtue of their diminished GABAergic inhibitory activity, disinhibition of reticular formation REM-on neurons and thus promote REM sleep.

6.4.1. PEPTIDES

Many peptides are colocalized with the neurotransmitter acetylcholine in LDT/PPT neurons; this colocalization likely also means they are coreleased with acetylcholine and may modify responsiveness to acetylcholine, as well as having independent actions. The peptide substance P is found in about 40% of LDT/PPT neurons, and, overall, more than 15 different colocalized peptides have been described. The role of these peptides in modulating acetylcholine activity relevant to wakefulness and sleep remains to be fully elucidated, but the colocalized peptide VIP has been reported by several different investigators to enhance REM sleep when it is injected intraventricularly.

6.5. REM-Off Neurons Suppress REM Sleep Phenomena

As discussed, REM-on neurons are those neurons that become active in REM sleep compared with slow-wave sleep and waking (Fig. 9) and likely act to promote REM sleep. Neurons with an opposite discharge time course that become inactive in REM sleep are called REM-off neurons. REM-off neurons are most active in waking, have discharge activity that declines in slow-wave sleep, and are virtually silent in REM until they resume discharge near the end of the REM sleep episode. This inverse pattern of activity to REM-on neurons and to REM sleep phenomena such as PGO waves has led to the hypothesis that these neurons may be REM-suppressive and interact with REM-on neurons in control of the REM sleep cycle, as illustrated in Fig. 9 and Fig. 14. This concept is indirectly supported by production of REM sleep from cooling (inactivating) the nuclei where REM-off neurons are found and by some *in vivo* pharmacologic studies. *In vitro* data have provided direct support for the inhibition of cholinergic LDT neurons by serotonin and norepinephrine, and *in vivo* unit recording and microdialysis experiments

have shown it is the REM-on neurons whose activity is suppressed by serotonin. The following classes of neurons are REM-off (see Fig. 10 for anatomy). *Norepinephrine-containing neurons* are principally located in the locus coeruleus, called the “blue spot” because of its appearance in unstained brain. *Serotonin-containing neurons* are located in the *raphe system* of the brain stem, the midline collection of neurons that extends from the bulb to the midbrain, with higher concentrations of serotonin-containing neurons in the more rostral neurons. *Histamine-containing neurons* are located in the posterior hypothalamus and are REM-off. This system has been conceptualized as one of the wakefulness-promoting systems, in agreement with drowsiness as a common side effect of antihistamines. Transection studies indicate, however, that the histaminergic neurons are not essential for the REM sleep oscillation. A mathematical and structural model of the occurrence of REM sleep based on interaction of REM-off and REM-on neurons, originally proposed by McCarley and Hobson, and revised by McCarley and Massaquoi, rather accurately predicts the timing and percentage of REM sleep over a night of human sleep and its variation with the circadian temperature rhythm and is the basis for Fig. 9. Figure 14 illustrates the structure of interaction of groups of neurons proposed to generate REM sleep. An area of intense current investigation is examination of why raphe and locus coeruleus activity declines over the sleep cycle and become nearly absent in REM sleep. One explanation is that the discharge activity of locus coeruleus/dorsal raphe nucleus (LC/DRN) neurons diminishes as a result of feedback inhibition from the recurrent inhibitory collaterals that are present in both LC and DRN neurons (illustrated as no. 3 in Fig. 14). This recurrent inhibition acts at $5HT_{1A}$ receptors in DRN and at α_2 receptors in LC. Although this recurrent inhibition is present, there is no clear evidence that it might be the causal agent in REM-off neurons turning off. A nonexclusive and likely possibility, illustrated as no. 4 in Fig. 14, is that GABAergic input, active during REM, inhibits DRN and LC neurons. GABA levels, as measured with *in vivo* microdialysis, are significantly increased in REM sleep compared with waking at both DRN and LC sites. Recent data suggest this might come from GABAergic neurons outside these nuclei, as microiontophoresis of GABA-A antagonist bicuculline restores tonic DRN discharge during REM sleep and retrograde labeling showed GABAergic (GAD-positive)

neurons from widely dispersed areas project to the DRN. The interrupted line in Fig. 14 (no. 5) from reticular formation to the GABAergic neurons indicates a possible reticular formation source of GABAergic input, an input supported by anatomic evidence. Other data indicate GABAergic input (labeled no. 6) may come from the more distant sites, including hypothalamus near the VLPO region important in non-REM sleep (the extended VLPO, source of the most dense projection), the substantia nigra reticular part, the ventral tegmental area, and the ventral pontine periaqueductal gray. However, unit recordings in these nonreticular areas have yet to identify neurons with the proper time course of activity to produce, through inhibition, the observed state-related decrease in DRN activity in non-REM and the virtual silence in REM sleep.

An alternative REM-on and REM-off model composed exclusively of GABAergic neurons has been proposed by Lu, Saper and co-workers. This is based on c-Fos expression data, lesions, and anatomic connectivity mapping, although cellular electrophysiologic data confirming the postulated REM-off and REM-on discharge characteristics have not yet been obtained. In this model, c-Fos data point suggests the presence of REM-off neurons in an arc of brain stem extending from the ventrolateral periaqueductal gray matter (vlPAG) and continuing laterally and ventrally in a reticular area termed the *lateral pontine tegmentum (LPT)*. It is postulated that LPT GABAergic REM-off neurons inhibit REM-on (by c-Fos criteria) GABAergic neurons in regions just ventral and dorsal to the LC. In turn, these GABAergic REM-on neurons are postulated to inhibit GABAergic REM-off neurons in the vlPAG-LPT, comprising a flip-flop switch arrangement in which each side inhibits the other. The issue of how REM sleep periodicity might come about in this flip-flop model is not clearly addressed. From a formal mathematical point of view, two mutually inhibitory populations will not produce periodic activity, such as the REM cycle. Some external input would be required to get two mutually inhibitory populations out of a state in which one inhibitory population predominates (the ecological analogy would be two populations of predators, where one would eventually devour the other, rather than the cycling observed in the prey-predator equations of the Lotka-Volterra model on which Fig. 9 is based). Moreover pre-REM neuronal activity in the brain stem is not an immediate flip-flop transition from SWS to REM but rather a gradual change as seen in Fig. 9.

7. OREXIN, NARCOLEPSY, AND THE CONTROL OF SLEEP AND WAKEFULNESS

An exciting development in 1999 was the discovery of the important role of lateral hypothalamic neurons containing the neuropeptide orexin (alternatively known as hypocretin) in behavioral state regulation and narcolepsy/cataplexy (see anatomic schematic in Fig. 15). Narcolepsy is a chronic sleep disorder that is characterized by excessive daytime sleepiness, fragmented sleep, and other symptoms that are indicative of abnormal REM sleep expression; these latter symptoms include cataplexy, hypnagogic hallucinations, sleep-onset REM periods, and sleep paralysis. Cataplexy consists of sudden attacks of bilateral atonia, especially in antigravity muscles, frequently with consequent collapse; these attacks are often provoked by emotion or excitement. Hypnagogic hallucinations consist of hallucinations upon falling asleep. Work by Mignot and co-workers indicated an abnormality in the gene for the orexin type II receptor was the basis of canine inherited narcolepsy, whereas Yanagisawa and co-workers found that orexin II receptor knockout mice (-/-) have increased REM sleep, sleep-onset REM periods, and also cataplexy-like episodes entered directly from states of active movement (Fig. 15). Recent confirmation in man of orexin's importance has been provided by evidence that narcoleptic humans often have undetectable levels of orexin in CSF and by a human postmortem study that found the number of orexin neurons reduced by 90%. As well as the control of wakefulness and sleep, orexins may have a neuromodulatory role in several neuroendocrine/homeostatic functions such as food intake, body temperature regulation, and blood pressure regulation.

7.1. The Orexin System

The orexins likely function as neurotransmitters as they are localized in synaptic vesicles and have neuroexcitatory effects on hypothalamic neurons. Orexin/hypocretin was identified by two independent groups. De Lecea and co-workers identified two related peptides from mRNA from hypothalamic tissue, which they named hypocretin-1 and -2. Sakurai and co-workers identified these same two peptides, which they termed orexin-A (=hypocretin-1) and orexin-B (=hypocretin-2) in a systematic biochemical search to find endogenous peptide ligands that would bind to G protein-coupled cell surface receptors that had no previously known ligand (orphan receptors). Orexin-A and -B are neuropeptides of

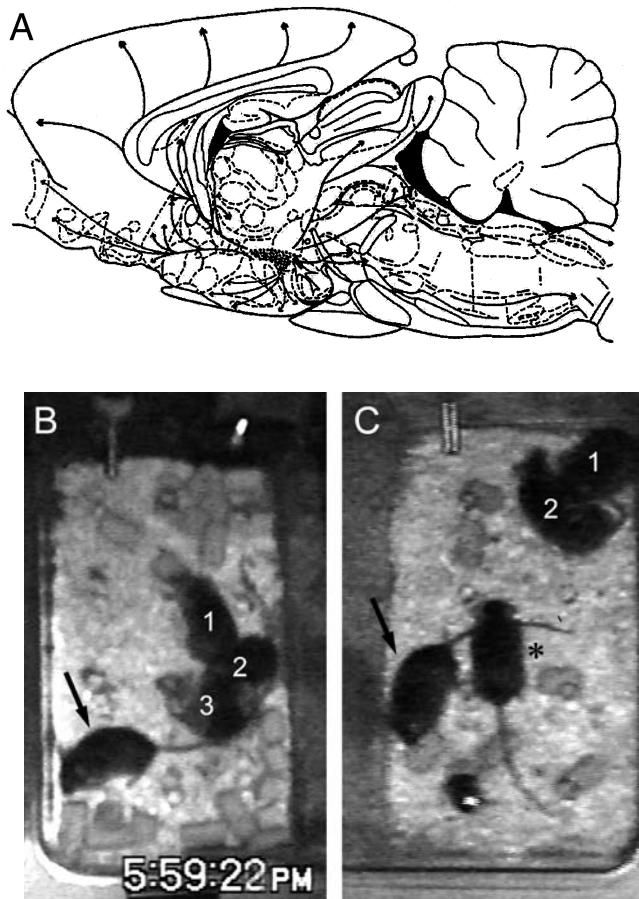


Fig. 15. (A) Schematic sagittal section drawing of location of orexin-containing neurons (dots in hypothalamus) and their widely distributed projection pathways in the rat brain. (Modified from Fig. 14 of Peyron C, Tighe D, van den Pol A, de Lecea L, Heller H, Sutcliffe J, Kilduff T. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J. Neurosci* 1998; 18:9996–10015.) (B) Digitally captured infrared video image of orexin knockout mice at 4 weeks of age. Note that one mouse (arrow) has completely fallen onto his side in a cataplexy episode (confirmed in other mice by EEG). The film shows the fuzziness (motion artifact) associated with body movement in behaving acting littermates designated 1 to 3. (C) Digitally captured infrared video image of orexin knockout mice at 4 weeks of age. Note that one mouse has fallen completely onto his side (arrow), and another is collapsed onto his ventral surface (asterisk). Littermates designated 1 and 2 are quietly sleeping in their usual corner of the cage. In both (B) and (C), the dark (active) phase onset was at 5:30 PM, and (C) was recorded at 8:26 PM. (Panels B and C reproduced with permission from Fig. 3 in Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki YY, Fitch TE, Nakazato M, Hammer RE, Saper CB, Yanagisawa M. Narcolepsy in orexin knockout mice: Molecular genetics of sleep regulation. *Cell* 1999; 98:437–451; a video of these episodes is available at <http://www.cell.com/cgi/content/full/98/4/437/DC1>.)

33 and 28 amino acids, respectively; they are derived from a single precursor protein.

Immunohistochemical studies reveal a distribution of orexin projections that is remarkable for the targeting of a number of distinct brain regions known to be involved in the regulation of sleep and wakefulness including both brain stem and forebrain systems. Orexin projections to forebrain include the cholinergic basal forebrain (see illustration in Fig. 5) and the histaminergic tuberomammillary nucleus (TMN). Brain-stem targets include the pontine and medullary brain-stem reticular formation, the cholinergic mesopontine tegmental nuclei (LDT/PPT), the locus coeruleus, and the dorsal raphe nucleus (see the brain-stem schematic in Fig. 10).

Two orexin receptors have been identified. Orexin-A is a high-affinity ligand for the orexin receptor type I (orexin-I), whose affinity for orexin-B is 1 to 2 orders of magnitude lower. The orexin receptor type II (orexin II) exhibits equally high affinity for both peptides. Currently, there are no ligands sufficiently specific for orexin I and II receptors to define their distribution. Of the brain regions involved in state control, only the dorsal raphe nucleus and the locus coeruleus appear to show a predominance of mRNA for type I receptors.

7.2. Orexin and the Control of REM-Related Phenomena

The knockout and canine narcolepsy data suggested that an absence of orexin or a defective orexin II receptor will produce cataplexy. What brain region(s) might mediate this effect? In the absence of an effective antagonist to orexin receptors, the author's laboratory decided to use antisense oligodeoxynucleotides (ODN) against the mRNA for orexin type II receptors, thereby producing a "reversible knockout" or "knockdown" of the type II orexin receptor. Spatial specificity was obtained by microdialysis perfusion of orexin type II receptor antisense in the rat pontine reticular formation (PRF) just ventral to the LC (but presumably not affecting the LC, which has predominantly type I receptors). This treatment, as predicted, increased REM sleep two- to threefold during both the light period (quiescent phase) and the dark period (active phase). Furthermore, this manipulation produced increases in behavioral cataplexy suggesting that the REM sleep and narcolepsy-related role of orexin is mediated via the action of orexin in the brain-stem nuclei that control the expression of REM sleep signs. Using short interfering RNAs (siRNA) to produce a knockdown of

the orexin pre-propeptide in the hypothalamus, the author's laboratory found a marked increase in the REM sleep during the normally active dark period in the rat, compatible with that hypothesis that orexin acts to suppress the REM oscillator during circadian-regulated active periods.

7.3. Orexin and the Control of Wakefulness

Orexin A has been shown to excite the noradrenergic neurons of the locus coeruleus, providing at least one documented mechanism by which orexin can promote wakefulness and suppress REM sleep. However, orexin is not always excitatory, and orexin has a variety of effects at the cellular level, both presynaptic and postsynaptic. The net effect of these actions on a particular brain circuit system physiology and consequent behavioral effects needs to be determined at the systems level for each brain region. The author's laboratory has recently found that microdialysis perfusion of orexin into the cholinergic basal forebrain of the rat produced a dose-dependent enhancement of wakefulness, with the highest dose producing more than a fivefold increase in wakefulness.

It is not yet certain whether orexin release is a function of the circadian cycle and/or a function of behavioral state, but initial data from a number of groups favor a model of circadian control of release. At the time of writing, the field of orexin research is one of intense activity. Orexin is particularly interesting to the sleep researcher and clinician because it affects both REM sleep and wakefulness and is likely closely linked to the human sleep disorder of narcolepsy.

8. MOLECULAR BIOLOGY OF SLEEP

An early round of studies focused on immediate early genes (IEG), such as c-fos, and found that, in a number of species, the expression of IEG is very low or absent during non-REM sleep but, as a rule, is very high when the animal is spontaneously awake or sleep deprived. To be noted as an exception is c-fos expression in the ventral preoptic area, where some cells express c-fos as a function of time asleep (see discussion in Section 4). Recently, other techniques have been used to obtain more specific indicators of which genes might be differentially expressed, including differential display and cDNA microarray technology. Interestingly Tononi et al. (2000) report that only a small subset (<0.01%) of genes have expression altered during the sleep cycle, and an even smaller subset is affected by long-term sleep deprivation. Wakefulness expression of IEG seems to be under

the control of the locus coeruleus. The mRNA transcripts of genes affected by wakefulness and sleep fall into three main groups: (1) genes resident in mitochondria, probably reflecting changes in energy demand during wakefulness and relatively short-term (3 h in the rat) sleep deprivation; (2) IEG and genes for transcription factors, perhaps related to plasticity; and (3) a heterogeneous group of other genes, including growth factors BDNF (brain-derived neurotrophic factor) and bone morphogenetic protein. This latter group showed more expression after long-term sleep deprivation (8 h in the rat), a pattern not seen in the first two groups. There is increasing interest in rest-activity cycles in lower animals, such as *Drosophila*, where an analysis of genetic expression is simpler than in higher organisms. Obviously, the molecular biology of sleep is in a phase of rapid advancement of knowledge, and its integration with the considerable body of knowledge concerning sleep mechanism appears to be an important future pathway for progress.

9. THE FORM OF DREAMS AND THE BIOLOGY OF REM SLEEP

REM sleep is strongly associated with dreaming, with about 80% of awakenings during the REM state producing a dream report. In experiments involving awakenings at random intervals throughout the night, 80% of all such randomly elicited dream reports have been found to occur in REM sleep. Those dreams that do occur in non-REM sleep have been found to be less vivid and intense than REM sleep dreams, suggesting they may represent a pre-REM state in which brain-stem neuronal activity is approximating that of REM sleep but the EEG has not yet changed.

Dreams have a long history of interest both in popular culture and in psychiatry. Sigmund Freud, writing before the presence of the biological state of REM sleep was known, suggested that dreams represented a symbolic disguise of an unacceptable unconscious wish (e.g., sexual or aggressive wishes); the purpose of the disguise was to prevent the disruption of sleep that would occur with consciousness of the undisguised wish. Today the activation of the neural systems responsible for REM sleep would seem to be a more accurate and simple explanation for the instigation of the dream state that is linked to the cyclic appearance of REM sleep. There remains the question, however, of why dreams have their own distinctive characteristics and are different from waking consciousness. One obvious hypothesis is that the

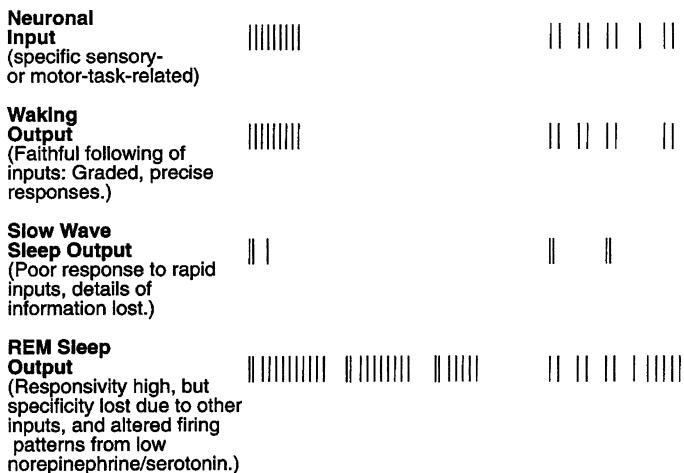


Fig. 16. Schematic of cortical neuronal processing during various behavioral states. The spikes represent neuronal discharge.

conscious states are different because the brain states differ. Figure 16 schematizes the “input-output” relationships in a cortical neuron during the various states of consciousness; this schematic is based on inferences from *in vivo* recordings in animal and *in vitro* experiments examining firing patterns as a function of membrane potential. This schematic suggests obvious differences in processing during the different behavioral states.

The *activation-synthesis hypothesis* proposed by Hobson and McCarley suggests that many of the characteristic formal features of dreams are isomorphic with (i.e., parallel) distinctive features of the physiology of REM sleep. By formal features is meant universal aspects of dreams, distinct from the dream content particular to an individual. As an example of a formal feature of a dream consider the presence of motor activity in dreams. At the physiologic level, it is known that motor systems, both at the motor cortex and at the brain-stem level, are activated during REM sleep episodes. Paralleling motor system activation at the physiologic level is the finding that movement in dreams is extremely common, with almost one-third of all verbs in dreams indicating movement and 80% of dreams having some occurrence of leg movement (a movement that was easily and reliably scored in dream reports).

Similarly, there is activation of sensory systems during REM sleep. The visual system is intensely activated in REM, and all dreams have visual experiences (and indeed these are one of the defining features of a dream). An important source of visual system activation during REM sleep is from the PGO waves. The activation synthesis theory suggests

that this intense activation of visual and other sensory systems are the substrate for dream sensory experiences. Supporting this theory is the rather frequent occurrence—about 9% of all REM sleep dreams—of dreams with intense *vestibular sensations* (i.e., dreams of flying, floating, falling, soaring, tumbling, etc.) easily relatable to the vestibular system functions of sensing position of the body in space and changes of position. The presence of dreams with vestibular sensations was highly atypical of the daytime sensory experience of the subjects whose dream reports were examined and is thus incompatible with any dream theory linked to a simple “recall” of previous experiences. Rather, the dream experience may reflect the intense REM sleep vestibular system activation, followed by its elaboration and synthesis into dream content. The final product, the dream, thus represents the synthesis of both the brain stem–induced motor and sensory activation with the particular memories and personality characteristics of the dreamer.

Lesion-induced release of REM sleep motor activity supports (1) the presence of neural commands for patterned motor activity in REM sleep and (2) a direct correspondence of the motor system commands and the subjective content of the dream. Activation of motor systems in REM can be observed in cats with a lesion of the muscle atonia zone of the pontine reticular formation and a consequent *REM sleep without atonia*, a state in which motor activity is released but all of the other signs of REM sleep are present. The failure of muscle atonia is also observed in a human disorder reported by Schenck and Mahowald and called *REM sleep behavior disorder*. In individuals with this disorder, the muscle activity observed has been found always to parallel the dreamed activity. This close linkage between the physiology and psychology of REM sleep and dreams supports the activation-synthesis hypothesis.

When the activation-synthesis hypothesis was first proposed, it aroused considerable controversy, perhaps because it seemed to threaten psychological interpretation of dreams. Although this theory clearly places instigation of the dream state as a concomitant of a basic biological rhythm, there appears, at least to the author, to be more than ample room for addition of personal characteristics in the process of synthesis of brain stem–instigated activation. For example, interpretations of Rorschach cards are rich sources of information on personality, although the images on the cards themselves were certainly not generated by psychologically meaningful mechanisms.

As more is learned about forebrain processing during REM sleep, a more complete understanding of the dream process will emerge. A series of fMRI and PET studies during sleep has increased our insight into sleep in man. In normal non-REM sleep there is a global decrease in cerebral blood flow, especially in brain stem, thalamus, and basal forebrain. In contrast, REM sleep is characterized by metabolic and hemodynamic evidence of activation of pons, thalamus, and basal forebrain. Of particular interest during REM sleep are the deactivation of the dorsolateral prefrontal cortex, perhaps related to absence of executive control and absence of a sense of control over one’s activities in the dream, and the increased amygdala and limbic system activation, perhaps related to emotions during dreams. These data thus suggest an isomorphism of dream emotion and a usual sense of lack of control over the course of the dream with the brain activation pattern. Also, as described in Section 10, many current theories postulate a role for this state in memory processing, and dreams may come by their unusual character as a result of the complex associations that are culled from memory during the REM sleep state.

10. FUNCTION(S) OF NON-REM AND REM SLEEP

There is little question about the importance of sleep to the organism. Perhaps the most dramatic evidence is the work of Rechtschaffen showing that rats die after 2 to 3 weeks of total sleep deprivation and after about 5 weeks of selective REM sleep deprivation. These numbers may be compared with the 16 days of survival with total food deprivation. Other factors arguing for the importance of sleep is its ubiquity among higher organisms, its evolutionary persistence despite its being maladaptive with respect to other key functions (food gathering, nurturing young, etc.), and its homeostatic regulation. As to the exact nature of its function(s), there are many theories but most have relatively little solid supporting data. Here we summarize some of the most plausible functional theories for each sleep phase.

10.1. Non-REM Sleep: A Time for Rest and Recovery?

10.1.1. REST THEORY

Neuronal recordings and brain metabolic studies indicate the presence of rest on the neural level as well

as the behavioral level during non-REM sleep. The data on adenosine provide rather strong support for non-REM sleep being a time of restoration of energy stores consumed during wakefulness.

10.1.2. BEHAVIORAL IMMOBILIZATION OR “OUT OF HARM’S WAY”

This theory suggests that sleep evolved as a way of arresting behavior at a time when it might not be advantageous, such as night activity in animals with poor night vision and vulnerability to predators.

10.1.3. CONSOLIDATION OF LEARNING

Recent data suggest learning is enhanced during non-REM sleep, and it is speculated that the in-rush of calcium during delta waves (see Section 5) is related to plastic changes. Evidence strongly suggests procedural motor and sensory memory (memory of “how to do” motor activity) is importantly dependent on non-REM sleep, whereas sleep’s relationship to declarative memory is less well understood. A related topic, that of brain plasticity during sleep, which may form the basis of memory consolidation, is currently under intense investigation. At the cellular level, there is now strong evidence that hippocampal long-term potentiation, thought to be an important substrate of learning-related plasticity, is impaired with sleep loss.

10.1.4. MAINTENANCE OF VIGILANCE AND COGNITION DURING WAKEFULNESS

It is common sense that we are more subjectively sleepier after sleep loss. What is now being investigated are the precise cognitive consequences of sleep loss. Sleepy individuals, including medical students and interns, are subject to transient microsleeps, with loss of consciousness and motor control, a loss that can be disastrous if one is driving home after a long stint at work or in the hospital. One of the more striking findings is that of great individual variability in humans in susceptibility to the deleterious effects of sleep loss, perhaps related to differences in brain biology, and perhaps analogous to the great variability of response to sleep loss in different (genetically homogenous) rodent strains. Discovering the cause of this variability is an important topic of current work.

10.2. REM Sleep: Does Its Activity Promote Growth and Development?

The following theories are not mutually exclusive. Indeed it seems a cogent argument that a complex behavioral state such as REM sleep may have multiple

functions, and, as for wakefulness, it may not be meaningful to speak of “the” function of REM sleep.

10.2.1. PROMOTION OF GROWTH AND DEVELOPMENT OF THE NERVOUS SYSTEM

The abundance of this metabolically and neuronally active state in the young argues for this hypothesis. Recent work has also indicated that REM-like activity in the brain stem may alter the activity of *immediate early gene* systems, such as c-fos, thereby suggesting a mechanism by which REM activity might affect DNA transcription and hence effect developmental and structural changes. Along this line of reasoning, the French scientist Jouvet has suggested that the stereotyped motor command patterns of REM sleep are useful in promoting epigenetic development of these circuits. Proteomic studies have indicated that sleep loss affects many structural elements of neurons, including the cytoskeleton, and proteins linked to synaptic function.

10.2.2. A CIRCUIT EXERCISE/MAINTENANCE FUNCTION IN THE ADULT

It is postulated that maintenance of neural circuits requires use, and that with increasing diversity of behaviors possible in more advanced animals, REM sleep serves as a “fail safe” mode for ensuring activation and consequent maintenance of sensorimotor circuits. Crick and Mitchison suggested that the REM sleep activity involves removal of unwanted, “parasitic” modes of neural circuit processing.

10.2.3. MEMORY PROCESSING

Memories may be consolidated and/or processed during sleep. Hippocampal neurons that encode spatial location and that are activated during wakefulness are preferentially activated in subsequent REM periods compared with the non-wake-activated neurons; the inference is that “memories” are being related to other brain information. Whereas this theory originally met with skepticism, evidence is now increasingly supporting this notion of replay during sleep of neuronal discharge sequences present during wakefulness.

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Disorders of Sleep

Gregory Cooper and Gerald Eichhorn

Although the exact role of sleep in humans and other animals has not been clearly determined, disruption of sleep can have serious consequences for the health of the affected individual. Disorders of sleep can result from a number of conditions, all with varied causes and associated symptoms. For example, one of the most common conditions resulting in disturbed sleep is obstructive sleep apnea syndrome. In this condition, patients experience either a relative collapse of the airway resulting in reduced airflow (hypopnea) or complete airway closure with resultant apnea. These episodes may occur hundreds of times per night with associated brief arousals and severely disrupted sleep continuity. The patient experiences significant and excessive daytime somnolence and may fall asleep frequently during the day, including while driving.

DISORDERS OF SLEEP MAY BE RELATED TO SLEEP STAGE

Other disorders result from disturbances during specific stages of sleep. Sleep is normally separated into stages 1 through 4 (non-REM sleep) and REM sleep. Stages 3 and 4 are sometimes combined under the heading of slow-wave sleep (SWS). Normal individuals will progress through the stages of sleep in a fairly predictable pattern beginning with stage 1 sleep. The initial period of REM sleep is usually experienced approximately 90 min after sleep onset. This cycle or progression is then repeated throughout the night. REM sleep, also sometimes termed *paradoxical sleep*, is characterized by rapid eye movements and a low-amplitude desynchronized, or activated EEG pattern. Interestingly, it is also marked by a suppression of skeletal muscle tone (muscle atonia) despite a highly

activated brain state. Dreaming is thought to occur primarily during REM sleep, and it is believed that this muscle atonia helps prevent the acting out of dreams in normal individuals.

REM behavior disorder (RBD) is a condition that typically begins in middle to older age individuals. It is characterized by a loss of the muscle atonia normally seen during REM sleep and a tendency to act out dreams. In some cases, individuals have sustained serious injuries including fractured vertebrae related to this activity.

NARCOLEPSY

Narcolepsy Results in Breakdown of the Separation Between REM Sleep, Other Sleep Stages, and Wakefulness

Narcolepsy is an interesting condition in which there appears to be a breakdown in the boundaries separating the various states of wakefulness and sleep as well as between the individual stages of sleep. This condition typically begins during the teen years or in early adulthood. However, there is often a significant delay of many years between symptom onset and correct diagnosis. The cardinal symptoms of narcolepsy are excessive daytime somnolence, cataplexy, sleep paralysis, and hypnagogic hallucinations.

Excessive daytime somnolence may result in involuntary napping throughout the day for periods ranging from minutes to greater than 1 hours. Patients may experience a sudden and irresistible urge to sleep and typically feel refreshed after these naps. Affected individuals may then be relatively refractory to further sleep attacks for 1 to several hours. For this reason, scheduled naps throughout the day are often recommended.

Cataplexy, in its most dramatic form, results in paralysis, typically induced by laughter or some other strong emotional stimulus. In practice, it is often seen as a relative weakness of the limbs, buckling of the knees, or even an episode of facial weakness. Symptoms are typically brief. Physiologically, cataplexy is associated with inhibition of the deep tendon reflexes and H-reflex similar to what is seen in REM sleep. Cataplexy can be considered a partial intrusion of the muscle atonia seen in REM sleep into wakefulness.

Sleep paralysis occurs upon falling asleep or with awakening. Patients are unable to move their limbs and may be unable to speak. Hallucinations are frequently associated with these periods. Paralysis typically lasts minutes.

Hallucinations associated with sleep onset are termed *hypnagogic hallucinations*. These may be visual or auditory. Some affected individuals have noted feelings of levitation, out-of-body experiences, or a sense of change in body part locations. It has been postulated that these sensations are related to decreased sensory feedback associated with motor inhibition of the spinal motoneurons seen during sleep paralysis or with REM sleep. All of these symptoms can be related to an abnormal intrusion of REM related activity into wakefulness or at sleep onset.

REM onset sleep is a hallmark of narcolepsy and is currently used as a standard for diagnosis. Patients with suspected narcolepsy are typically evaluated with an overnight sleep study, or polysomnogram, in addition to a mean sleep latency test (MSLT) the following day. The MSLT consists of a series of five 20-min opportunities to nap at scheduled times throughout the day. Normal, nonsleepy individuals will have an average sleep or nap latency of greater than 10 min. However, narcoleptics or other pathologically sleepy individuals typically have latencies of less than 5 min. In addition, narcoleptics will typically progress into REM sleep within a few minutes of falling asleep on at least two naps. Normal individuals do not typically progress into REM sleep during a brief nap of 20 min or less.

Pharmacology of Narcolepsy

The pathophysiology underlying narcolepsy is still poorly understood, though significant strides have been made in the past few years. Much of what is known pharmacologically derives from observations of responses to medications in patients with narcolepsy and from the study of canines with narcolepsy. It has been demonstrated that medications that inhibit adrenergic uptake are anticitaplectic. These agents have

also been shown to inhibit REM sleep. Stimulant medications have been used for decades to treat the associated excessive daytime somnolence. These agents seem to work by blocking dopamine reuptake and thereby increasing dopaminergic transmission within the brain. Medications acting by this mechanism promote wakefulness but do not affect cataplexy. A role for the cholinergic system and cholinergic hypersensitivity has also been postulated. However, this has not led to any useful treatments thus far.

Canine Narcolepsy Results from Mutations in the Hypocretin Receptor (Hcrtr2) Gene

The genetic mutation responsible for canine narcolepsy observed in both Dobermans and Labradors was identified in the late 1990s. An exon-skipping mutation in the gene encoding a G protein-coupled hypocretin receptor (*Hcrtr2*) was demonstrated. A knockout mouse model for orexin (hypocretin), which is the ligand for this receptor, demonstrates abnormalities analogous to narcolepsy. Hypocretin-containing neurons are located in the dorsolateral hypothalamus. Partly related to this localization, these proteins were initially thought to be related to appetite regulation and were named orexin. It is now believed, however, that the orexins or hypocretins have only a minor role in appetite and a much more significant role in sleep regulation. Hypocretin-containing neurons project to monoaminergic brain-stem nuclei, including the locus coeruleus, and to cholinergic pontine neurons within the pontine reticular formation. The wake-promoting effects of the hypocretin system may be mediated through these aminergic and cholinergic systems, as well as histaminergic projections from the tuberomamillary nucleus.

Interestingly, approximately 90% of narcoleptic patients with cataplexy have undetectable levels of hypocretin in the cerebrospinal fluid. It has been speculated that narcolepsy may be a neurodegenerative disease affecting hypocretin-containing neurons of the dorsolateral hypothalamus. This could explain the development of symptoms, over time, in patients who initially had normal sleep patterns. It has also been speculated that the loss of these neurons could result from an autoimmune mechanism. In support of this notion is the correlation of narcolepsy with specific HLA haplotypes. However, no strong pathologic support for an inflammatory process has been found. This could be a result of the relatively long interval between development of narcolepsy and autopsy. Patients with narcolepsy do not have

shortened life spans and may live with this condition for several decades.

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CLINICAL CORRELATION: THE APHASIAS AND OTHER DISORDERS OF LANGUAGE *GREGORY COOPER, GERALD EICHHORN, AND ROBERT RODNITZKY*

APHASIC DISORDERS ARE USUALLY CAUSED BY LESIONS OF THE LEFT HEMISPHERE
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1. INTRODUCTION

We will use the term *higher brain functions* to refer to the operations of the brain that stand at the pinnacle of evolution and that, in many respects, are unique to humans. Verbal communication, the capacity to “think in the future,” and the ability to hold multiple tracks of complex information “online” at the same time are examples of complex mental functions subserved by various structures in the human brain. The “higher” capacities of the human brain can also be captured under the terms *cognition* and *behavior*. Cognition comprises capacities such as intellectual function, memory, speech and language, complex perception, orientation, attention,

judgment, planning, and decision-making. Behavior is the manifestation of these cognitive functions and also subsumes the important domain of emotional processing and regulation. Behavior is influenced and guided by another facet of higher brain function, namely, *personality*, which describes the psychological makeup, traits, and response styles that typify a person’s behaviors across a range of situations and circumstances.

Most contemporary neuroscientists view *brain functions* as synonymous with *mind functions*, even if we do not yet know all, or even many, of the details of how mental operations are served by neural machinery. In fact, it remains to be seen whether we will ever be able to account for all human behavior in neural terms, and some might even consider this an unlikely potentiality. Nonetheless, breakthroughs in neuroscience over the past couple of decades have

provided more and more compelling evidence pointing to the conclusion that brain facts and mind facts are one and the same. A basic premise behind the ideas presented in this chapter is that there are orderly, predictable relationships between neural operations, on the one hand, and cognitive and behavioral capacities, on the other. Such relationships are a product of both genetic endowment and environmental influences, to be sure, and individual differences abound in virtually every cognitive and behavioral capacity that one can consider, but even so, we assume that there are neurobiological forces that ground, constrain, and otherwise delimit the ways in which brain processes subserve mind processes.

To give an example of our thinking along these lines, consider the model we have proposed for the acquisition, storage, and retrieval of conceptual and lexical knowledge, that is, the semantic information and names for all manner of concrete entities, actions, and other concepts we come to know. We have proposed that at least three types of neural structures facilitate the process of recognition and retrieval of specific entities, including (1) structures that support conceptual knowledge, (2) those that support lexical retrieval, and (3) intermediary structures that broker between the two. The organization of these structures in higher-order cortices is evolutionarily adaptive and operates systematically, producing constraints on the brain's anatomic design that are both macroscopically similar and microscopically flexible across individuals.

The theory posits that large-scale sectors of the brain are organized within *convergence regions* of functional similarity. Convergence regions can themselves be broken down into smaller functional units known as *convergence zones*, which consist of microcircuits of neuron ensembles, and are the principle neural substrates of *dispositions*, or the implicit, nonconscious mechanisms by which mental *images* are constructed, recalled, executed, and regulated. Images, in this context, refer to explicit mental patterns of sensory activation of any type (visual, auditory, tactile), which can be experienced either consciously or outside of one's level of awareness. Images are flexible and modifiable within dispositions, which are continuously shaped by learning experiences both internal and external to the individual. Thus, the theory predicts that convergence regions will be functionally equivalent across individuals, but that the convergence zones activated by various dispositions will vary depending upon the specific task demands of the mental images being processed and the previous learning experiences of the individual.

We turn now to a discussion of specific brain-behavior associations.

2. BRAIN AND BEHAVIOR ASSOCIATIONS

2.1. History

The systematic study of brain-behavior relationships can be traced back to a number of landmark observations, beginning nearly a century and a half ago. In the 1860s, the surgeon and physical anthropologist Paul Broca reported on a patient who developed an inability to produce speech after damage to the left front part of the brain. The discovery led to the suggestion—at the time quite startling, but something now accepted as a basic principle of neuropsychology—that humans speak with the left side of the brain. Some 10 years later, the neuropsychiatrist Carl Wernicke reported a complementary finding: damage to the posterior part of the left hemisphere rendered patients unable to comprehend speech while leaving speech production relatively unaffected. And in this same general era, John Harlow reported on the case of Phineas Gage, a young man who developed a bizarre and striking impairment in personality and social conduct after an accident in which an iron bar was propelled through the front part of his brain, destroying prefrontal cortex bilaterally.

Other historical developments were also centered on key case studies. In 1957, Scoville and Milner reported on the patient who came to be known as H.M., who developed severe and permanent anterograde amnesia (learning impairment) after bilateral resection of the mesial temporal lobes, performed to control intractable epilepsy. Neuropsychological studies of H.M. yielded a number of key breakthroughs in the understanding of the neural basis of memory and focused attention on the role of the mesial temporal region—especially the hippocampus—in memory. And shortly after the middle of the 20th century, studies by Roger Sperry, in collaboration with Joseph Bogen and Michael Gazzaniga, sparked interest in the dramatic differences between the two hemispheres of the brain. These investigators studied “split-brain” patients, who had undergone separation of the two hemispheres for control of seizures. Specifically, the *corpus callosum*, the large bundle of fibers that connects the left and right hemispheres, was surgically cut, so that the left and right hemispheres were no longer in communication. Careful studies of these patients revealed that the two hemispheres retained two more or less separate modes of consciousness, one in the left hemisphere that was language-based and operated in sequential,

analytical style, and one in the right hemisphere that was spatially based and operated in gestalt, holistic style. Modern cognitive neuroscience has confirmed many of these earlier findings.

In the past couple of decades, a number of additional important cases have been reported. For example, our laboratory has described a modern-day “Phineas Gage” type patient, known as E.V.R., who developed a profound impairment in social conduct and personality after bilateral damage to the ventromedial prefrontal cortex. Another patient, known as Boswell, developed one of the most severe amnesia syndromes that has ever been reported after bilateral damage to both the mesial and lateral sectors of the

temporal lobes. Boswell cannot learn any new declarative information, and he cannot recall anything more than a few shreds of information from his past. Interestingly, another patient, known as Clive Wearing, sustained extensive damage to the mesial temporal and inferior frontal lobes due to encephalitis—he has both a profound learning impairment and history of abnormal emotion regulation. Another patient known as S.M. cannot recognize emotional facial expressions or learn new associations between salient events and strong emotional responses, due to bilateral damage to the amygdala. A timeline of these and other important cases can be found in Table 1.

Table 1
Timeline of Landmark Case Studies in Cognitive Neuroscience

Date	Discovery	Contributors
circa 1861	A patient with damage to the left frontal operculum has a profound speech output impairment, leading to the inference that we speak with our left frontal lobe	Paul Broca
circa 1874	A patient with damage to the posterior portion of the left temporal lobe is unable to comprehend speech, illustrating that language production and comprehension are served by different brain areas	Carl Wernicke
circa 1868	Case study of Phineas Gage illustrates the importance of the frontal lobes in personality and social functioning	John Harlow
circa 1909	Patients with bilateral damage to the posterior parietal cortex are reported to manifest optic ataxia, optic apraxia, and simultanagnosia	Rezső Bálint
circa 1947	Two patients with acquired prosopagnosia are described. The area of brain damage is later discovered to be the fusiform gyrus in the temporal lobe.	Joachim Bodamer
circa 1957	Case study of patient H.M. shows that the medial temporal lobes are critical for anterograde memory	William Scoville
circa 1960s	Studies of “split brain” patients with severing of the corpus callosum show that the brain’s hemispheres process information independently and very differently	Brenda Milner Roger Sperry Joseph Bogen Michael Gazzaniga
circa 1970s	Experiments with patient K.C. indicate that episodic memory differs from other types of memory, such as procedural and semantic memory	Endel Tulving
circa 1980s	Observations with patient J.B.R. illustrate that category-specific recognition and naming deficits result from damage to the temporal lobes	Elizabeth Warrington Tim Shallice
circa 1985	Patient E.V.R. confirms and extends findings from Phineas Gage—the ventromedial prefrontal cortex is involved in personality, social conduct, decision-making, and executive functioning	Antonio Damasio Paul Eslinger Daniel Tranel
circa 1989	Investigations with patient Boswell highlight the role of the mesial and lateral temporal lobes for learning	Antonio Damasio Daniel Tranel Hanna Damasio
circa 1995	Patient S.M. highlights the role of the amygdala in fear, emotion, and classic conditioning	Ralph Adolphs Daniel Tranel

Many new breakthroughs in cognitive neuroscience continue to be discovered through clinical observations of case studies. Often, the discoveries made through these observations can then be tested empirically through the use of the lesion method, which is one of the time-honored, quintessential research methodologies used in the study of the brain and behavior. Using the lesion method, scientists can compare the performances of brain-damaged patients with focal, stable, and circumscribed lesions to normal, healthy individuals on a specific measure of a construct. For example, scores on a face-naming test can be compared between patients with brain damage to the left temporal pole and normal, healthy volunteers. Observed differences between these two groups can then be attributed to the damaged neural tissue in the lesion group, which in this case has led to the inference that the left temporal pole is important for naming faces. Because the lesion method relies on inferences drawn from performance deficits of patients with damaged brains, it is limited by the possibility that the brain-behavior relationships identified by this method could operate differently in normal, healthy individuals. Thus, cognitive neuroscience also relies on modern neuroimaging techniques, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) to provide a glimpse into the normal mind at work.

2.2. Lateral Specialization: Left Versus Right

The pioneering discoveries of Broca and Wernicke, and the pathbreaking work of Sperry and colleagues, led to the establishment of what has become one of the most robust principles in neuropsychology; namely, that humans have left-hemisphere specialization for language. This principle applies to nearly all right-handed persons (about 99%) and to the majority of left-handers (about 70%). Moreover, this principle holds irrespective of the mode of sensory input—verbal material apprehended through the visual (as in reading) or auditory (as in hearing spoken language) modalities is processed by the left hemisphere; and it holds irrespective of the mode of output—both spoken speech and written language production are subserved by left hemisphere structures.

For many years in the early history of neuropsychology, the right hemisphere was thought to be the “silent” or “minor” hemisphere, because it did not participate to any great extent in language. Thus, in early conceptualizations of the differences between the left and right hemispheres, the prevailing notion

was that the left hemisphere was the major or *dominant* side, and the right hemisphere was the minor or *nondominant* side. This attitude reflected an emphasis on language—because language is a highly observable and uniquely human capacity, it received the most attention from neurologists and neuropsychologists and was considered the quintessential human faculty. For many decades, the right hemisphere was thought to contribute little to higher-level cognitive functioning. Lesions to the right hemisphere typically did not produce language disturbances, and it was often concluded that the patient had lost little in the way of higher-order functions after right-sided brain injury. We know now that this is far from true—although the right hemisphere has an entirely different type of specialization, it has cognitive and behavioral capacities that are every bit as important as those of the left hemisphere.

As the field evolved, it became clear that each hemisphere was dedicated to certain cognitive capacities, and the notion of *dominance* gave way to the idea of *specialization* (in fact, one rarely encounters the terms *dominant/nondominant* or *major/minor* any more, in reference to the two cerebral hemispheres). That is, each hemisphere was specialized for certain types of cognitive functions (Table 2). As noted, early work had already established the role of the left hemisphere in language function, and subsequent investigations confirmed this conclusion. By contrast, the right hemisphere is specialized for *nonverbal processing*, and it handles information such as complex visual patterns (e.g., faces, geographical routes) or auditory signals (e.g., music) that are not coded in

Table 2
Functional Dichotomies of Left and Right Hemispheric Dominance

<i>Left side</i>	<i>Right side</i>
Verbal	Nonverbal
Serial	Parallel
Analytic	Holistic
Controlled	Creative
Logical	Pictorial
Propositional	Appositional
Rational	Intuitive
Social	Physical

Adapted from Benton, A.L. (1991). The Hecaen-Zangwill legacy: Hemispheric dominance examined. *Neuropsychology Review*, 2, 267–280.

verbal form. The right side of the brain is also specialized for the mapping of emotions, that is, patterns of bodily sensations that are linked to feelings such as happiness, anger, and fear. Another right-hemisphere capacity concerns the perception of our bodies in space, in intrapersonal and extrapersonal terms. For example, an understanding of where our limbs are in relation to our trunk and where our body is in relation to the space around us is under the purview of the right hemisphere.

2.3. Longitudinal Specialization: Anterior Versus Posterior

Another useful organizational principle for understanding brain-behavior relationships is an *anterior* and *posterior* distinction. The major demarcation

points are the *Rolandic (central) sulcus*, which is the major fissure separating the frontal lobes (anteriorly) from the parietal lobes (posteriorly), and the *sylvian fissure*, which forms a boundary between the temporal lobes (inferiorly) and the frontal and parietal lobes (superiorly). These landmarks, and the major lobes of the brain, are illustrated in Fig. 1.

As a general principle, the posterior regions of the brain are dedicated to *perception*, that is, the apprehension and intake of information from the world outside. (The “world outside” really refers to two domains: the world outside that is outside our bodies and brains, and the world outside that is outside our brains but inside our bodies. The latter, the *soma*, comprises the smooth muscle, the viscera, and other bodily structures innervated by the central nervous system.) The primary

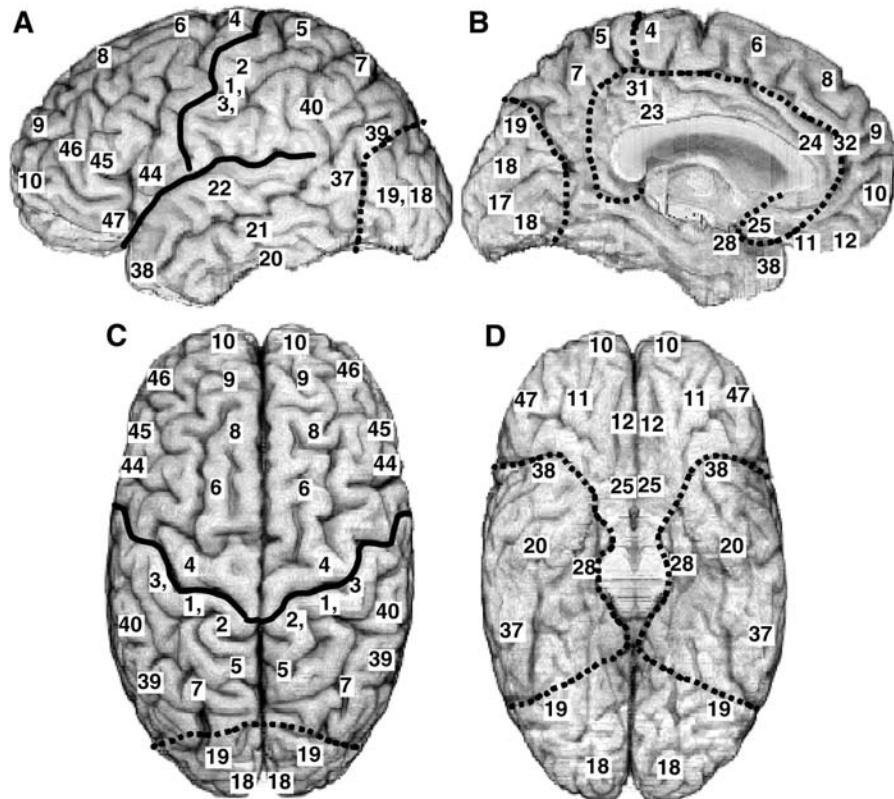


Fig. 1. (A) Lateral, **(B)** mesial, **(C)** superior, and **(D)** inferior views of the brain, depicting major demarcation points and Brodmann areas (numbers). The left hemisphere is shown in the **(A)** lateral and **(B)** mesial views; the mappings would be the same on the right hemisphere. **(C)** In the superior perspective, the left hemisphere is on the left, and the right hemisphere is on the right; **(D)** the sides are reversed in the inferior perspective. The Rolandic (central) sulcus runs in a roughly vertical fashion from the top of the brain (just behind area 4) down to an intersection with the sylvian fissure, just behind area 44. The sylvian fissure (lateral sulcus) runs in a roughly horizontal fashion, just above area 22. The main lobes of the brain are demarcated: the *frontal lobe* is anterior to the Rolandic sulcus and superior to the sylvian fissure; the *parietal lobe* is posterior to the Rolandic sulcus and superior to the sylvian fissure; the *occipital lobe* is behind the dotted line that separates areas 37 and 39 from areas 19 and 18; the *temporal lobe* is below the sylvian fissure. Also, a region commonly referred to as the *limbic lobe* includes the cingulate gyrus (areas 24 and 23), and areas 25, 26, 27, and 28.

sensory cortices for vision, audition, and tactile perception are in the posterior sectors of the brain, in the occipital, temporal, and parietal regions, respectively.

By contrast, anterior brain regions generally comprise effector systems specialized for the execution of behavior. For example, the primary motor cortices are located in the strip of cortex immediately anterior to the Rolandic sulcus (area 4). The motor area for speech, known as *Broca's area*, is in the left frontal operculum (areas 44 and 45). The right-hemisphere counterpart of Broca's area, in the right frontal operculum, is important for executing stresses and intonations that infuse speech with emotional meaning (e.g., prosody). A variety of "executive functions," such as judgment and decision-making, and the capacity to construct and implement various plans of action, are associated with structures in the frontal lobes. Thus, it is a useful heuristic to consider the anterior part of the brain as comprising a variety of effector systems and the posterior part as comprising a variety of perceptual systems. (This is a heuristic, and there are exceptions, e.g., the sense of smell, which is subserved by the pyriform cortex in the posterior orbital prefrontal region.)

3. THE OCCIPITAL LOBES

3.1. Primary Visual Cortex

The occipital lobes are situated in the posterior part of the hemispheres, and they can be subdivided into primary visual cortex and visual association cortices (Fig. 2). The primary visual cortices are composed of area 17, located primarily in the region directly above and below the calcarine fissure in the mesial aspect of the hemispheres. This region is dedicated to *form vision*, and damage here produces blindness in the corresponding visual field. The system is

wired in a crossed fashion, in both the vertical and horizontal dimensions. Hence, visual information from the hemispace to the right of the vertical meridian is perceived with the left visual cortex, and information from the left hemispace is perceived with the right visual cortex. Similarly, information from visual space above the horizontal meridian reaches visual cortex below the calcarine fissure, and information from visual space below the midline reaches cortex above the calcarine fissure.

Other features of the visual world are processed in or near the primary visual cortices. The processing of color, for example, is strongly linked to the lingual gyrus, immediately below primary visual cortex on the inferior bank of the calcarine sulcus. *Depth perception* (i.e., stereopsis) and *motion perception* are associated with cortices in and near the superior component of the primary visual region, in an area known as the cuneus.

3.2. Visual Association Cortices

The visual association cortices include areas 18 and 19 on the lateral and mesial aspects of the hemispheres. Area 37, and the posterior parts of areas 20 and 21 in the inferior and ventral banks of the temporal lobes, are also dedicated primarily to the processing of visual information. The visual association cortices, which communicate with primary visual cortex posteriorly and with more anterior regions in the temporal and parietal lobes through a series of extensive feedforward and feedback connections, are specialized for progressively higher-order aspects of visual processing.

3.3. The Ventral and Dorsal Visual Systems

Visual system characteristics can be conceptualized along anatomic and functional lines comprising

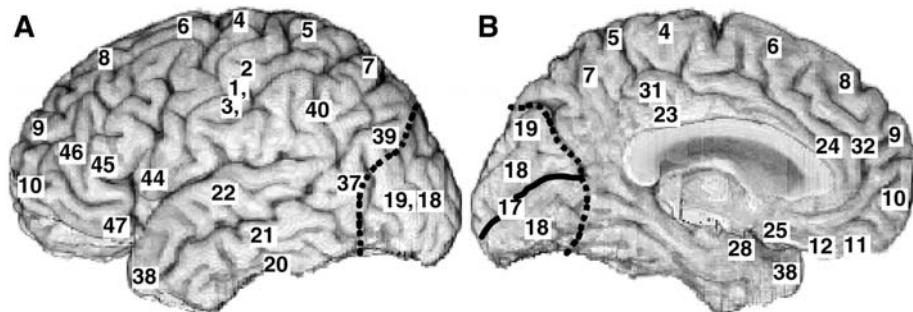


Fig. 2. The major subdivisions of the occipital lobes, mapped on (A) lateral and (B) mesial views of the left hemisphere (the mappings would be the same in the right hemisphere). The calcarine fissure is a major landmark, separating the ventral (inferior) visual system from the dorsal (superior) visual system. The primary visual cortex is formed by the superior and inferior banks of the calcarine region, in area 17. Areas 18 and 19 make up the visual association cortices.

two distinct subsystems: a ventral system and a dorsal system. These are referred to as the “what” and “where” systems, respectively. Consistent with its dedication to the “what” aspects of stimuli, the ventral system processes primarily featural information such as shape, color, and texture. For example, the ability to perceive and assign meaning to orthographic symbols (i.e., reading) is associated with the region of cortex and white matter in the lower part of visual association cortex in the left hemisphere. In the right hemisphere, the ventral visual association cortices are specialized for the registration and decoding of nonverbal patterns, such as the holistic perception and recognition of faces. The ventral occipitotemporal cortices are also important for retrieving conceptual knowledge regarding various concrete entities (e.g., recognition of animals, fruits/vegetables, tools/utensils, musical instruments, and other categories). There is remarkable specialization within these systems. For example, recognition of animals is subserved by a system that includes cortices in the right mesial occipital/ventral temporal region and the left mesial occipital region, whereas recognition of tools/utensils is subserved by a system that includes cortices in the left occipital-temporal-parietal junction.

The dorsal or “where” system processes primarily the features of motion, depth, and the position of stimuli in space. That is, superior parts of the visual association cortices are important for deriving meaningful information from motion and depth. The anterior part of this region, which overlaps with the posterior part of the superior parietal region, is specialized for visuospatial capacities that relate to the placement of external stimuli in space and to the tracking of those stimuli when they are in motion. This region is also important for the accurate mental assemblage of extrapersonal space and the subsequent placement and location of oneself in that space. For example, the ability to know where your body is in relation to the chair on which you are sitting and to guide your arm down to a book to turn the page depends on association cortices in the upper parts of the occipital and posterior parietal regions. The upper part of the visual system is important for attending to multiple visual elements concurrently. When watching a politician delivering a speech from center stage, flanked on both sides by other dignitaries, we may be aware of the speech giver and the person just to the left of the podium who is having trouble staying awake. The capacity to do this is known as simultaneous visual perception.

4. THE TEMPORAL LOBES

The temporal lobes can be subdivided into three sectors, according to the type of specialization the regions have for different cognitive operations (Fig. 3).

4.1. Superior Sector: Primary Auditory Cortex and Auditory Association Cortices

Heschl’s gyrus (areas 41 and 42), which is buried in the depths of the sylvian fissure in the posterior aspect of the temporal lobes (Fig. 3), contains primary auditory cortex that is crucial for the basic perception of auditory information. The system is relatively crossed, so that auditory information from the right ear is sent primarily to left Heschl’s gyrus, and information from the left ear is sent primarily to right Heschl’s gyrus. However, this decussation is incomplete, and a significant amount of information is perceived ipsilaterally (i.e., by primary auditory cortex on the same side as the ear). Interestingly, information can be “forced” into more complete crossing using a special paradigm known as *dichotic listening*. In this task, auditory information is presented to each ear simultaneously. If the information varies sufficiently between the left and right ears (e.g., if the word *science* is input to the left ear and the word *professor* is input to the right ear), the auditory system will be driven in such a way that the subject will perceive the word *science* with right auditory cortex and the word *professor* with left auditory cortex. When verbal material is presented in dichotic listening paradigms, the right ear (i.e., left brain) shows a relative advantage (i.e., perceives information first and more strongly) over the left ear. By contrast, if nonverbal material is presented (e.g., music), the left ear (right brain) shows a relative advantage over the right ear. This pattern is in keeping with the overall verbal-left, nonverbal-right hemispheric specialization.

The posterior third of the superior temporal gyrus (posterior area 22) contains important auditory association cortices (Fig. 3). On the left, this region comprises the heart of what is known as *Wernicke’s area*, specialized for decoding aural verbal information, such as deciphering the meaning of speech input. The right side is specialized for nonspeech auditory information, such as environmental sounds, musical melodies, timbre, and prosody. In general, the left auditory association cortices are specialized for the perception and decoding of *temporal* components of auditory information, that is, information pertaining to timing, the sequential aspects of auditory signals, the pace of information

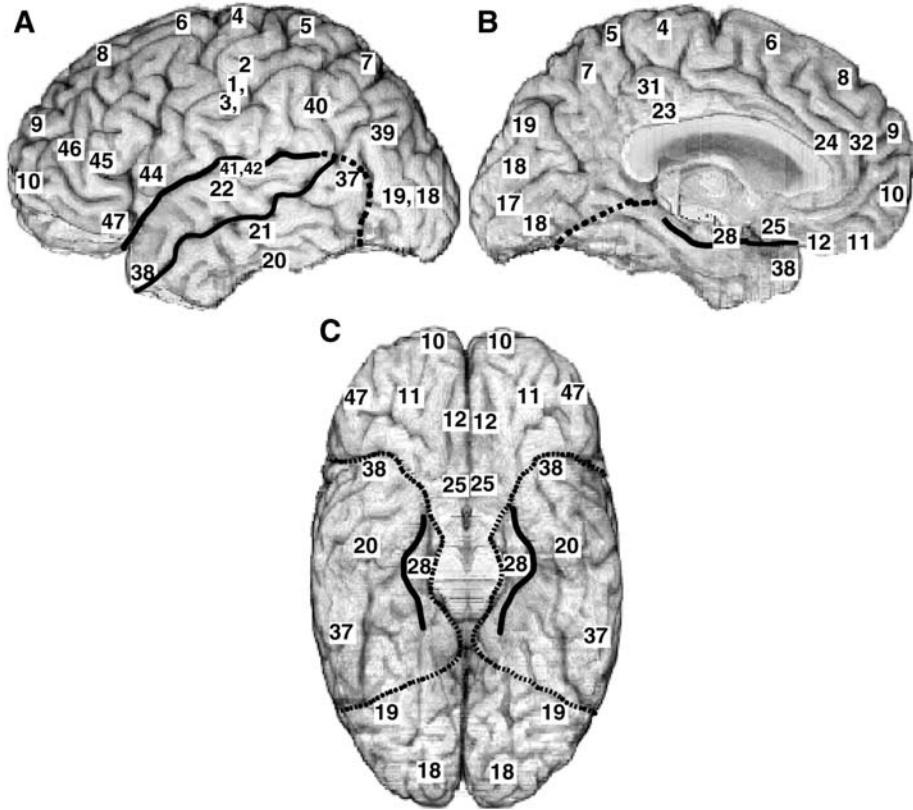


Fig. 3. Three major subdivisions of the temporal lobes are depicted on **(A)** lateral and **(B)** mesial views of the left hemisphere (the mappings would be the same in the right hemisphere). An inferior view of these regions is shown in **(C)** (where the left hemisphere is on the right). The temporal lobes are bounded overall by the sylvian fissure superiorly and by the junction with the parietal and occipital lobes posteriorly (*dotted line* in **A**). The primary auditory cortices (areas 41 and 42) are buried in the depths of the sylvian fissure; the auditory association cortices in the posterior superior temporal gyrus (area 22) surround this region. The lateral/inferior sector is made up of areas 21, 20, 37, and 38. The mesial sector is made up of area 28 and other limbic structures (hippocampus, amygdala) in the mesial part of the temporal lobes (**B, C**).

delivery (i.e., cadence), and the duration of signals and intervals between sounds. The right auditory association cortices are specialized for *spectral* information, that is, the pitch (i.e., fundamental frequency) of signals and their spectral complexity (i.e., harmonic structures).

4.2. Lateral and Inferior Sector

The lateral and inferior parts of the temporal lobe are comprised by areas 37, 20, 21, and 38 on the lateral and inferior aspects of the hemisphere (Fig. 3). The posterior parts of the second, third, and fourth temporal gyri formed by areas 37, 20, and 21, are strongly linked to the higher-order decoding of visual information. As alluded to above in the discussion of the ventral occipital region, the cortices in the ventral occipital and posterior inferotemporal regions are critical for face recognition and for visual recognition of other entities such as animals, fruits and vegetables,

and tools and utensils. On the left side, the more anterior parts of the inferotemporal region are specialized for the retrieval of lexical items that denote various entities, including common nouns such as the names of animals and fruits and vegetables. Progressively more anterior parts of the system on the left are concerned with progressively more unique items, and in the temporal polar region (area 38), there is a specialization for the retrieval of proper nouns, that is, unique lexical entries that denote items that constitute a class of one (such as names of people and places). Actually, evidence suggests that these regions contain important intermediary units that broker between the retrieval of conceptual knowledge (knowing what things are) and the retrieval of names (knowing what things are called). The intermediary units do not contain the words themselves; rather, the word assembly is performed by language-related structures in the perisylvian region.

The inferior and anterior temporal sectors in the right hemisphere play an important role in the retrieval of nonverbal information from retrograde memory, that is, the retrieval of knowledge that was acquired prior to the onset of a brain injury. This stands in distinction to the learning of new information, which depends on mesial temporal structures (see later). Remarkably, patients with damage to anterior lateral and ventral temporal structures, but not to mesial structures, can develop a pattern of amnesia that involves severe disruption of retrograde memory, but nearly complete sparing of anterograde memory; that is, the patients can learn new information, but they cannot retrieve information from their past. The right anterior temporal region works in conjunction with structures in the prefrontal sector to subserve retrieval of knowledge related to one's autobiography.

Structures in the right anterior temporal region appear to play an important role in the *recognition* of unique entities (e.g., familiar persons and landmarks) in a manner akin to the role of the left anterior temporal region in retrieving names for such entities. Thus, when the right temporal pole is damaged, patients develop impairments in the retrieval of conceptual knowledge for familiar persons. This finding has been corroborated by functional imaging studies, which have demonstrated activation of this region when subjects are identifying familiar persons and landmarks. These results are also consistent with the importance of anterior and lateral aspects of the right temporal lobe in the retrieval of retrograde memories. Together with interconnected right prefrontal cortices, the right anterolateral temporal region is important for the retrieval of unique, factual memories.

4.3. Mesial Sector

The mesial temporal lobe comprises the amygdala, hippocampus, entorhinal and perirhinal cortices, and the anterior portion of parahippocampal gyrus not occupied by the entorhinal cortex (Fig. 3). These structures play a crucial role in memory, especially learning of new information (anterograde memory). Below, a brief summary of the roles of the hippocampal complex and amygdala is presented.

4.3.1. HIPPOCAMPAL COMPLEX

The hippocampus and the adjacent entorhinal and perirhinal cortices are referred to as the *hippocampal complex*. The components of the hippocampal complex are highly interconnected by means of recurrent neuroanatomic circuits. In turn, the hippocampal

complex is extensively interconnected with higher-order association cortices located in the temporal lobe. Those cortices receive signals from the association cortices of all sensory modalities and also receive feedback projections from the hippocampus. Structures in the hippocampal complex thus have access to, and influence over, signals from virtually the entire brain. Anatomically, the system is in a position to create integrated records of various aspects of memory experiences, including visual, auditory, and somatosensory information. In a general sense, the principal function of the hippocampal complex is the acquisition of new factual knowledge. There are two hippocampal complexes, one in the left hemisphere and one in the right. Anatomically, the two are roughly equivalent, but there are major differences in their functional roles. Specifically, the two hippocampal complexes are specialized for different types of material in a manner that parallels the overall functional arrangement of the brain, namely, left-verbal and right-nonverbal.

The landmark report by Scoville and Milner described how patient H.M. became severely amnesic after bilateral mesial temporal lobe resection for control of intractable seizures. This observation established the mesial aspect of the temporal lobes, and the hippocampus in particular, as unequivocally linked to memory, specifically, to the acquisition of new information (i.e., *anterograde memory*). Also, research has established a fairly reliable relationship between the extent of damage to the hippocampal system and the severity of amnesia that develops. More extensive mesial temporal damage, involving the perirhinal cortex and parahippocampal gyrus in addition to the hippocampal region and entorhinal cortex, tends to produce a proportionate increase in the severity of amnesia.

With respect to the nature of the amnesia associated with hippocampal damage, several relationships have been firmly established. First, there is a consistent relationship between the side of the lesion and the type of learning impairment. Specifically, damage to the left hippocampal system produces an amnesic syndrome that affects verbal material (e.g., spoken words, written material) but spares (at least relatively) nonverbal material; conversely, damage to the right hippocampal system affects nonverbal material (e.g., complex visual and auditory patterns) but spares (at least relatively) verbal material. For example, after damage to the left hippocampus, a patient may lose the ability to learn new names but remain capable of learning new faces and spatial

arrangements. By contrast, damage to the right hippocampal system frequently impairs the ability to learn new geographical routes.

A second point is that the hippocampal system does not appear to play a role in the learning of perceptuomotor skills and other knowledge known as *nondeclarative memory*. Patient H.M., for example, can learn new perceptuomotor skills, even though he has no recall of the situation in which the learning of those skills took place. We have reported similar findings in other patients with bilateral mesial temporal lobe damage. In fact, not only can such patients acquire perceptuomotor skills at a normal level, but they can retain those skills for many years after the initial learning, despite the fact that they cannot recall the circumstances of the learning situation. Thus, the role of the hippocampus in memory is principally for acquiring declarative knowledge (i.e., facts, faces, names, and other information that can be “declared” and brought into the mind’s eye).

Finally, the hippocampus and related mesial temporal structures do not appear to be as crucial for the retrieval of previously learned information (*retrograde memory*). As noted above, other structures in anterior and lateral temporal cortices appear to be the critical repositories of retrograde memory. By contrast, the hippocampus system, although it is crucial for the acquisition of information, does not seem to be necessary for the retrieval of information, once that information has been consolidated and stored. In short, the hippocampal system plays a time-limited role in memory—it is critical for acquisition but not for long-term storage and retrieval. (We acknowledge that this is an ongoing and unresolved scientific issue, however, and newer studies have tended to support some role for the hippocampus in retrieval of previously acquired knowledge. There is much to be learned about how different facets of memory are subserved by different brain structures.)

4.3.2. AMYGDALA

The amygdala plays an intriguing role in memory. The amygdala is important for the acquisition and expression of *emotional memory* but not for neutral memory. Specifically, the amygdala contributes critically to the potentiation of memory traces for emotional stimuli during their acquisition and consolidation into long-term declarative memory. These findings are in accord with other evidence indicating that the amygdala is important for the recognition of emotion in facial expressions, especially fear, and in the processing of other information that has emotional significance. Also, it has been shown that the

amygdala is important for classic conditioning of autonomic responses. One study found that a patient with circumscribed bilateral amygdala damage was able to acquire declarative knowledge normally but was impaired in acquiring conditioned autonomic responses; a patient with circumscribed bilateral hippocampal damage (but with intact amygdala) showed the opposite pattern. These findings have led to the idea that the amygdala is important for processing stimuli that communicate emotional significance in social situations; specifically, the amygdala may orchestrate patterns of neural activation in disparate sectors of the brain that would encode both the intrinsic, physical features of stimuli (e.g., shape, position in space) and the value that certain stimuli have to the organism, especially emotional significance.

5. THE PARIETAL LOBES

The parietal lobes, situated posterior to the Rolandic sulcus and superior to the sylvian fissure, comprise a heterogeneous collection of primary sensory and association cortices (Fig. 4). Basic somatosensory perception, including perception of touch, vibration, and temperature, takes place in the strip of cortex formed by the postcentral gyrus (areas 3, 1, and 2). The postcentral gyrus contains a neural map of the body’s sensory space known as the *sensory homunculus*, which is topographically and disproportionately organized such that body areas associated with enhanced tactile analysis (hands, lips, face), are more extensively represented. A secondary somatosensory area (SII) is in the inferior parietal operculum, and this region may play an important role in higher-order tactile perception (e.g., recognition of objects from touch). In the right hemisphere, SII is important for the mapping and interpretation of emotional states. The inferior parietal lobule, which includes areas 40 and 39, is closely linked to the auditory modality and is strongly connected to nearby auditory association cortices in the temporal lobe. The superior parietal lobule, made up of areas 7 and 5, is linked to the visual modality and is strongly connected to visual association cortices in the occipital lobe. However, the region formed by the transition zone between the occipital, temporal, and parietal cortices on the lateral aspect of the hemisphere is highly heteromodal and is specialized for polymodal sensory integration, such as integration of visual, auditory, and somatosensory signals.

On the left, the inferior parietal lobule is involved in language functions. For example, fibers from the

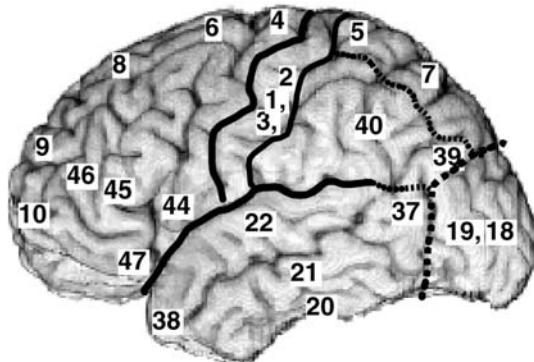


Fig. 4. The major subdivisions of the parietal lobe are depicted on a lateral view of the left hemisphere (the mappings would be the same in the right hemisphere). The primary somatosensory cortex is made up of areas 3, 1, and 2. The superior parietal lobule includes areas 5 and 7. The inferior parietal lobule is made up of areas 40 (supramarginal gyrus) and 39 (angular gyrus).

arcuate fasciculus, which is the fiber tract connecting Broca's area to Wernicke's area, travel through the angular gyrus in the left inferior parietal lobe. The ability to repeat verbatim words, digits, or sentences, for example, depends on intact parietal opercular cortices and underlying white matter. This function requires accurate perception of auditory information, the retrieval of matching information from one's store of acoustic records, and the triggering of anterior motor cortices to produce the information. Inferior parietal cortices on the right side play a significant role in self-perception, the placement of a person's body in space, and in the mapping of physical and emotional states. The ability to direct attention to the external and internal milieu, for example, depends critically on right inferior parietal cortices. The right parietal region is also crucial for many aspects of visuospatial processing, such as perceiving and deciphering complex spatial patterns and comprehending and manipulating spatial knowledge (e.g., planning how to pack a large amount of luggage into your car trunk). Visuoconstructional skills (e.g., drawing complex figures, or architecture-type endeavors) are also related to the right parietal region. Patients with damage to the right parietal lobe have been known to manifest a variety of deficits, including neglect for the left side of their bodies and neglect for visual stimuli in the left visual field.

6. THE FRONTAL LOBES

The frontal lobes comprise a vast expanse of cortex and white matter anterior to the Rolandic sulcus (Fig. 5). The frontal lobes make up nearly half of the

entire cerebral mantle, and they represent the highest level of neural evolution. The cognitive operations mediated by the frontal lobes, such as foresight, complex decision-making, and social conduct, stand at the zenith of evolution of mental processes. The frontal lobes can be subdivided into several functional units that have distinctive behavioral correlates (Fig. 5). Four major subdivisions are reviewed here.

6.1. Motor and Premotor Region

Immediately anterior to the Rolandic sulcus is the strip of motor cortex (area 4) that mediates basic motor activity of all parts of the body. Anterior to this is area 6, which together with area 44 comprises the premotor region. The cortex in area 6 also participates integrally in motor behavior, as a sort of motor "association" cortex—it is involved with the planning and initiation of motor behaviors. The premotor region has access to complex information from all major sensory modalities, and it participates in the formation of a plan for motor activity in response to particular stimulus configurations and provides the "impetus" to set the plan into action.

The frontal operculum includes areas 44, 45, and 47. On the left side, areas 44 and 45 of this region constitute *Broca's area*, which is the main speech output center; that is, the region responsible for motor aspects of linguistic expression. In the right hemisphere, the frontal opercular region plays a role in expressive prosody, that is, the infusion of speech with various intonations that add emotional coloring to the content.

6.2. Dorsolateral Prefrontal Region

The dorsolateral prefrontal region is composed of the cortices and white matter formed by the lateral expanses of areas 8, 9, 46, and 10 (Fig. 5). This region plays an important role in many types of higher-order intellectual behavior. Recent investigations, especially those using functional neuroimaging techniques such as PET and fMRI, have provided some consistent clues regarding the functions of the dorsolateral prefrontal region, and some of the most notable findings are summarized below.

Various types of high-level intellectual abilities appear to depend on the dorsolateral prefrontal region. Examples include mental operations that require the retrieval and manipulation of information, especially when there is a demand for creativity and originality. To the extent that such operations involve verbal material and the use of language

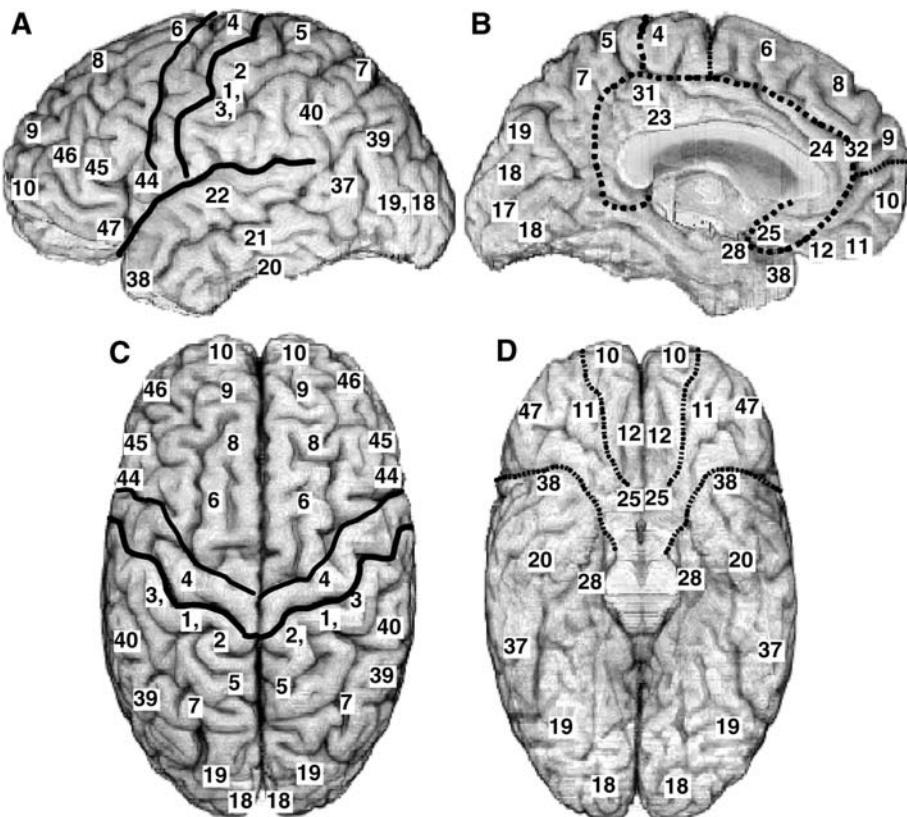


Fig. 5. The major subdivisions of the frontal lobes are depicted on (A) lateral and (B) mesial views of the left hemisphere (the mappings would be the same in the right hemisphere). (C) Superior and (D) inferior mappings are also depicted; in (C), the left hemisphere is on the left, and in (D), the left hemisphere is on the right. The primary motor cortex is formed by area 4. The premotor region includes areas 6 and 44. In the left hemisphere, areas 44 and 45 make up Broca's area. The large expanse of cortex composed primarily of the lateral aspects of areas 8, 9, 46, and 10 forms the dorsolateral prefrontal region. The superior mesial prefrontal region is formed by the mesial aspects of areas 6, 8, 9, and the upper part of area 32. The ventromedial prefrontal region is made up of areas 12, 11, 25, and the lower mesial parts of areas 32 and 10.

symbols, the left dorsolateral region has the dominant role. When operations depend on and require the use of nonverbal information, the right dorsolateral region takes the lead. Consider the following two neuropsychological tasks, which are analogous in their demand for creative, flexible mental production but which vary in the use of verbal versus nonverbal mediation. In one, known as *verbal fluency*, a person is required to generate as many words as possible that begin with a particular letter of the alphabet (e.g., *f*) within a specified time limit. In the other task, known as *design fluency*, the person is asked to generate as many unique geometric designs as possible within a particular time constraint. Both tasks require retrieval, manipulation, and execution in a manner that depends critically on flexible, creative thought processes, and the dorsolateral prefrontal region is an important neural substrate for performance of tasks such as these.

Another important function subserved by the dorsolateral prefrontal region is *working memory*. Working memory refers to a brief window of mental processing—of the order a minute or two—during which a limited amount of information is held in a sort of mental scratchpad, and operations can be performed on it. Working memory is used to bridge temporal gaps, so that we can perform operations on material that is being held “in-mind” but is no longer existent in our perceptual space. For example, when we look up a phone number and run it through our minds a couple of times, and then cross the room to the telephone and dial the number, the ability to hold the number in mind during the time it takes to cross the room and dial the number is an example of working memory. The two hemispheres are specialized for working memory in a manner that parallels overall hemispheric specialization; that is, the left dorsolateral region is dominant for verbal working

memory (e.g., remembering a list of grocery store items), and the right dorsolateral region is dominant for spatial working memory (e.g., remembering a geographical route after asking for directions in a strange town).

The dorsolateral prefrontal region is also associated with the capacities of judging the *recency* and *frequency* of events. For example, consider the following question, which calls for a recency judgment: “When was the last time you talked to your father on the telephone?” The answer may be anywhere from a few minutes ago up to many years ago, and to find the answer, your brain will engage a memory search that requires complex and simultaneous activations of various interrelated memories. Now consider a judgment of frequency: “How many times did the temperature go above 100 degrees last summer, in your hometown?” Again, answering such a question requires a complex activation and search of memory that depends on dorsolateral prefrontal structures. And as with many other brain-behavior relationships, there appears to be some hemispheric specialization for recency and frequency judgments, with the left dorsolateral prefrontal region being more important for verbally coded information, and the right being more important for visuospatial information. The dorsolateral prefrontal sector has also been linked to other types of “cognitive estimations” that require rough approximations rather than retrieval of rote knowledge, such as guessing the average number of publications that full professors have, or the average number of weeks that a hit song stays in the “Top Forty.”

6.3. Superior Mesial Prefrontal Region

On the mesial surface of the cerebral hemispheres, there is a region of the frontal lobes composed of the supplementary motor area (SMA; mesial area 6) and the mesial parts of areas 8, 9, and 32 (Fig. 5). Together with the anterior part of the cingulate gyrus (i.e., area 24), this region is closely linked to emotional behavior and to basic drive states associated with motivation and arousal. The SMA plays a crucial role in motivating the execution of motor behaviors. Without the input of the SMA, a person may never get past the stage of intention to act. The same principle applies to speech production—the SMA plays an important role in the basic drive to produce speech, and when the SMA is dysfunctional, patients tend to remain mute, even though they retain the basic capacity for speaking.

The superior mesial prefrontal region also is important for a variety of emotional and motivational behaviors. This region, for example, participates in behaviors that include elements of obsessiveness, compulsiveness, anxiety, and introversion or extroversion. The maintenance of an adaptive and optimal state of arousal and alertness is linked to this region. Having one’s surveillance systems “set” in such a way as to maximize the relation between energy expenditure and the detection of important external and internal events is a function that is linked to the superior mesial prefrontal region. Having a sense of how long to persevere on a particular line of goal-directed behavior or when to desist and seek an alternate track is another example. The spontaneous, automatic selection and implementation of an appropriate emotional response to a particular stimulus configuration is linked to the superior mesial prefrontal region and anterior cingulate. Examples include having a mirthful response to a good joke, having a feeling of sorrow on being told of a sad event, or having a response of empathy while watching a child endure a painful social learning experience.

In a related vein, recent studies have linked the anterior cingulate region to a set of functions that have to do with *response selection* and *conflict resolution*. For example, when confronted with situations that call for selecting the best course of action from among several closely matched alternatives, the brain shows increased activity in the anterior cingulate region. Similarly, when trying to decide between two conflicting response options, each of which carries with it certain rewards and punishments, the anterior cingulate comes into play in a critical fashion. A related capacity is *error detection*, which also appears to depend intimately on the anterior cingulate cortex. This refers to our ability to monitor our behavior and responses so that we can detect—and correct either on-line or post hoc—various errors that are made.

6.4. Ventromedial Prefrontal Region

The ventromedial prefrontal region includes the orbital frontal cortex, formed by areas 11 and 12, and nearby regions in the ventral and lower mesial aspects of the frontal lobes, including parts of areas 25, 32, and the mesial aspect of areas 10 and 9 (Fig. 5). The ventromedial prefrontal region comprises a functional unit that plays a key role in judgment, planning, and decision-making and in a wide range of behaviors that can be grouped under the heading of social conduct. The mediation of these functions is done in part through the use of emotional states as guideposts for behavior, so that we use our emotions

and feelings, both consciously and nonconsciously, to help make complex decisions in a way that is in our best long-term interests. These emotional guideposts have been termed *somatic markers*.

Social conduct, when executed in such a way as to maximize one's personal gain in the short-term and long-term, and in the overall best interests of society in general, depends on the rapid selection of appropriate courses of action, often in highly ambiguous situations. Such selection requires several component processes, including bringing on-line a range of viable potential courses of action, the assignment to each of these a degree of likelihood in terms of its short-term and long-term consequences for reward or punishment, and consideration of the implications of such courses of action in the context of the person's current position in the world. This type of decision-making process is intimately linked to the ventromedial prefrontal region. Damage to the ventromedial prefrontal region produces severe disturbances in social contact while sparing most basic components of cognition.

Anatomically, the ventromedial prefrontal cortices are well situated for these functions. They receive signals from a large range of neural structures engaged by perception, including external information from vision, audition, and olfaction, and internal somatic information from skeletal and visceral states. In turn, the ventromedial prefrontal cortices are a source of projections from frontal regions toward central autonomic control structures; also, the ventromedial cortices receive and reciprocate projections from hippocampus and amygdala. Thus, the ventromedial cortices are in a position to form conjunctive records of concurrent signals hailing from external and internal stimuli, and they can also activate somatic effectors.

The ventromedial prefrontal region also appears to play a role in *prospective memory*, which refers to the capacity of "remembering in the future." There are numerous everyday situations that require this capacity (e.g., when we must remember to place a phone call at a certain time of day or remember to keep a preset appointment). The manner in which the brain actually mediates this function remains largely unknown, but it may use a mechanism whereby a somatic marker can be affixed to a particular stimulus array in such a way that when that array (or parts thereof) becomes extant in perceptual space, the marker is triggered, which in turn triggers a "feeling to act" (i.e., a reminder that we are supposed to execute a particular task).

7. SUBCORTICAL STRUCTURES

Many subcortical structures participate in various aspects of higher-order cognition and behavior, although subcortical structures are not, generally speaking, as directly linked to such functions as is the cerebral cortex. Such structures include the basal ganglia, thalamus, cerebellum, and brain-stem nuclei. Two of these—the basal ganglia and thalamus—are reviewed here.

7.1. Basal Ganglia

The basal ganglia are a collection of gray matter nuclei deep in the brain, composed mostly of the *striatum*, which includes the caudate nucleus and the putamen, and the *pallidum*, which includes the globus pallidus. The striatum and pallidum play basic roles in motor behavior, particularly in the automatic execution of highly learned motor patterns. For example, the kinds of motor behaviors that are called into play when a person rides a bicycle, dances, skates, or skis, depend on the basal ganglia.

In addition to their role in automatic motor behaviors, the striatum and pallidum also make important contributions to some aspects of higher-order cognition and behavior. The left caudate nucleus, especially the region known as the "head" of the caudate, plays an important role in language function, paralleling to some extent the roles played by interconnected cortical regions in the perisylvian region of the left hemisphere. The left putamen has a role in speech articulation, specifically, in the implementation of strings of phonemes in the motor system in a smooth, seamless manner that allows fluent, canonical articulation. On the right side, the striatum plays an important role in prosody. It is also involved in various visuospatial functions, including visual perception and visual construction, in a manner generally analogous to the cortical regions of the right hemisphere.

The striatum and pallidum are important for the acquisition of information that falls under the designation of nondeclarative knowledge. In contrast with declarative knowledge (the learning of which is mediated by mesial temporal structures, as discussed earlier), nondeclarative knowledge cannot be brought to mind for conscious inspection, and instead typically depends on a motor output in order to be instantiated. For example, the learning of perceptuomotor skills such as typing, playing the guitar, hitting a golf ball, or ice skating depends on the basal ganglia. The learning of "habits" (i.e., a tendency to respond automatically and consistently to a particular stimulus

configuration) is also probably linked to the basal ganglia. In addition, there is evidence that the learning of “gut-level” likes or dislikes for certain stimuli may depend on the striatum.

7.2. Thalamus

The thalamus has a critical role in the transmission of motor and sensory information from lower subcortical systems to various regions of the cortex. Evidence also supports a role for the thalamus in higher-order cognition and behavior, especially in attention, memory, and language. The thalamus contains the reticular nuclei, which have an important role in the network of neural structures responsible for arousal and attention. The capacity to direct attention to a particular stimulus and to attend to it sufficiently to allow the selection of an appropriate response are linked to basic arousal and attentional mechanisms mediated in part by the reticular nuclei.

The thalamus has a left-right specialization that mirrors that of the cortex. Structures in the left thalamus are geared more for verbal material, and structures in the right thalamus are geared more for nonverbal, spatial material. The left anterior thalamus plays a role in language that is related to comprehension and production aspects of linguistic function. The anterior part of the thalamus, which contains important pathways linking the thalamus to the hippocampus and amygdala (especially the mammillothalamic tract and the ventroamygdalofugal pathway), has been linked to material-specific learning capacities; the left is important for verbal information, and the right is important for nonverbal information. There are midline thalamic nuclei, including the medial dorsal nucleus, which have an important role in memory. In general, the thalamus is more important for anterograde memory than for retrograde memory and for learning declarative types of knowledge as opposed to nondeclarative types of knowledge.

8. CLOSING REMARKS

The past couple of decades have witnessed an unparalleled growth in our understanding of how higher cognitive and behavioral functions are subserved by different brain structures. This expansion owes much to the power of new imaging techniques, beginning with the structural methods that arrived in the 1970s (CT) and 1980s (MRI), and continuing with the functional methods that have begun to dominate the recent literature in cognitive neuroscience (PET,

fMRI). These tools have provided powerful windows into the operation of the human brain and mind and have led to many new discoveries regarding brain-behavior relationships. At the same time, the development of sophisticated neuropsychological experimentation has allowed the design of elegant experiments that can isolate the basic constituents of various mental processes. And no less important are concurrent advances on the theoretical front, which have provided explanatory and predictive frameworks that have helped shape some of the most interesting questions in cognitive neuroscience and have helped fuel the rapid explosion of new studies in this area.

One would be remiss, though, not to sound a cautionary note here: the exciting new advances in cognitive neuroscience and in the understanding of brain-behavior relationships can lure the naïve student down a path of “neophrenology.” According to would-be neophrenologists, the notion that most or even all mental functions can be assigned to discrete brain regions is not only acceptable, but also is seen as the main objective of modern cognitive neuroscience. The fact is, for virtually any cognitive or behavioral function that one can imagine, the degree of variance accounted for by neuroanatomy is still far short of a 100%; in most cases, in fact, it is more of the order 10% to 30%. This leaves a huge amount of room for many other factors, including those that have to do with individual differences, learning history, and the interaction of cognition and personality. This is not to say that science will never be able to account for more than it does now, insofar as brain-behavior relationships are concerned, but it is to say that we must remain cautious and critical in our evaluation of the new explosion of studies that claim to have localized this or that function to a particular brain region.

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The Aphasias and Other Disorders of Language

*Gregory Cooper, Gerald Eichhorn
and Robert Rodnitzky*

Language is the mechanism by which communication is achieved through the use of specific sounds or symbols. In the clinical realm, it is extremely important to differentiate this process from that of *speech*, the co-ordinated motor mechanism that allows the utterance of sound. Either language or speech can be abnormal with total preservation of the other, although in some clinical circumstances, both are involved simultaneously. Conceptual abnormalities of production or understanding of speech or language are referred to as *aphasia*, and mechanical abnormalities in the motor production of speech are known as *dysarthria* or *anarthria*.

APHASIC DISORDERS ARE USUALLY CAUSED BY LESIONS OF THE LEFT HEMISPHERE

Language dominance is located in the left hemisphere in approximately 95% of right-handed persons and in approximately 66% of those people who are left-handed. Because most of the population is right-handed, a useful clinical principle is that the left hemisphere must be considered dominant for language in all persons until proven otherwise. In some circumstances, it is critical to determine the hemisphere in which language dominance resides with absolute certainty. For example, in patients undergoing surgical removal of a brain tumor or surgical excision of a portion of the temporal lobe as a treatment for epilepsy, the surgeon must know whether cortical structures in the hemisphere being operated on can be ablated without fear of inducing aphasia. Several techniques can be used to determine hemispheric dominance for language. Positron

emission tomography (PET) can be used to identify the cortical areas activated during speech, although its accuracy is still questioned. The most widely used and time-honored technique involves the injection of an anesthetizing drug, first into the carotid artery that perfuses the right hemisphere, and then into the carotid artery that perfuses the left hemisphere of the brain. The drug produces transient aphasia when injected into the artery perfusing the dominant hemisphere, but it has no effect when injected into the nondominant side. This technique is called the Wada test.

SEVERAL FORMS OF BRAIN PATHOLOGY CAN CAUSE APHASIA

Damage to the speech area of the dominant hemisphere may be caused by stroke, tumor, infection, trauma, or neurodegenerative conditions. Generally, the more rapid the development of the damage (e.g., sudden stroke), the more severe the aphasia. The severity of language dysfunction is also related to the extent of the damage. Regardless of the form of brain pathology, the age of the affected person may be a modifying factor. Dominant hemisphere pathology acquired in infancy or childhood does not usually cause aphasia, reflecting the plasticity of the brain at that age.

SEVERAL FACETS OF LANGUAGE MUST BE ASSESSED IN EVALUATING AN APHASIC DISORDER

Suspicion of an aphasic disorder usually develops as the examiner is listening to a patient's ordinary conversational or *propositional speech*. There may

be abnormalities in the rhythm and inflection of speech (e.g., *dysprosody*), in the use of proper word sequence or connecting words (e.g., *agrammatism*), in naming objects (e.g., *anomia*), or in repetition. In addition, words or sounds may be incorrectly substituted (e.g., *paraphasias*). The substitution of an incorrect sound (e.g., “spoot” for spoon) is known as a *phonemic paraphasia*. The substitution of a word related in meaning (e.g., fork for spoon) is known as a *verbal paraphasia*. When the substitution in a word is so severe as to render it unrecognizable (e.g., “sporaker” for spoon), it is referred to as a *neologism*. A person whose language is characterized by agrammatic speech, containing many neologisms and rendering it virtually incomprehensible, is said to have *jargon aphasia*.

APHASIA CAN BE DIVIDED INTO FLUENT AND NONFLUENT TYPES

Nonfluent aphasia typically results from dysfunction of the anterior portion of the speech area. Broca's aphasia, resulting from a lesion of the posterior portion of the inferior frontal gyrus, is the classic example of a nonfluent aphasia. In this condition, speech is hesitant and effortful, and the affected person appears frustrated. Connecting words such as articles and conjunctions are omitted, resulting in agrammatic and telegraphic speech, but the retention of appropriate nouns and verbs preserves meaning. Instead of “I want to see the doctor,” the nonfluent, Broca aphasic may utter “want, see, doctor.” In this form of aphasia, comprehension of language is largely intact. Because of the proximity of Broca's area to the motor cortex, most Broca aphasics also suffer from significant weakness on the right side of the body.

Fluent aphasia is characterized by a normal or even increased output of words. Prosody and construction are often normal, but speech contains many paraphasic errors. This form of aphasia is typically caused by a lesion in the posterior speech area. A lesion of the posterior third of the superior temporal gyrus gives rise to Wernicke's aphasia, the classic aphasia of the fluent type. In this aphasia, comprehension of language is distinctly impaired. Unlike Broca aphasics, who are frustrated by their language impairment, Wernicke aphasics show little awareness of their deficit, but after a time can become paranoid and agitated, perhaps related in part to an inability to communicate their needs and to a verbal isolation caused by impaired comprehension. Unlike Broca's aphasia, there is typically no weakness associated

with Wernicke's aphasia, because the causative lesion is usually far removed from the motor cortex. In fact, these patients may have an isolated aphasia without other associated neurologic signs or symptoms. For these reasons, patients with a Wernicke's aphasia have occasionally been misdiagnosed as having a psychiatric illness.

REPETITION IS USUALLY ABNORMAL IN APHASIA CAUSED BY LESIONS IN THE PERISYLVIAN REGION

Broca and Wernicke aphasics manifest abnormalities of repetition. This tends to be true of any aphasia resulting from a lesion involving perisylvian structures. Aphasias resulting from lesions distant from this region are characterized by intact repetition and are known as the transcortical aphasias. A lesion just anterior or superior to Broca's area results in language dysfunction similar to Broca's aphasia with the exception that repetition is intact. This is known as a *motor transcortical aphasia*. Similarly, a lesion just posterior to Wernicke's area results in a fluent aphasia with intact repetition known as a *sensory transcortical aphasia*. Persons for whom impaired repetition is the predominant abnormality are said to have *conduction aphasia*. The lesion is considered to be in one of the two loci, the supra marginal gyrus or the area encompassing the primary auditory cortices, insula, and underlying white matter, especially the arcuate fasciculus.

DISORDERS OF WRITTEN LANGUAGE

Impaired production and comprehension of written words is another common form of language disturbance. Impairment in production of written language is called *agraphia*. Agraphia almost always accompanies aphasia of spoken language. The writing abnormality may take the form of misspelling, agrammatism, or imperfectly constituted letters and words. Agraphia is such a common accompaniment of aphasia that its absence in a patient apparently afflicted with aphasia raises serious doubt about the diagnosis. Isolated agraphia without other disorders of language sometimes occurs when the dominant angular gyrus is damaged.

Abnormal comprehension of written words is called *alexia*. Acquired alexia, typically associated with agraphia, may result from lesions in a variety of locations, but damage to the dominant parietal lobe has traditionally been believed to be the most

common cause of this combination of deficits. *Alexia without agraphia* is a distinct and relatively uncommon syndrome. It results from infarction of the dominant (typically left) occipital lobe and the splenium of the corpus callosum. In this circumstance, visual language information is prevented from gaining access to the language areas of the left hemisphere from the left occipital lobe, which is infarcted, and from the intact right occipital lobe, which has been disconnected from the dominant hemisphere by destruction of the splenium of the corpus callosum, and therefore the crossing axons carrying visual information from the opposite hemisphere. Because the parietal language areas are spared, agraphia is conspicuously absent in this syndrome. Prominent loss of vision in the right half of the visual field is a usual accompaniment of this syndrome because of the involvement of the primary visual cortex on the left.

Developmental dyslexia refers to impaired development of reading and writing skills relative to that expected, based on overall intelligence. Typically, it becomes apparent in the school-age child. Intellectual functions often are not impaired, but affected children are sometimes mistakenly believed to be

mentally dull until the specific isolated language dysfunction is discovered. Although there are no gross abnormalities of the brain in these persons, postmortem and MRI studies have shown that developmental dyslexics often exhibit subtle structural abnormalities. For example, they may lack the expected interhemispheric asymmetry in a portion of the temporal lobe known as the planum temporale. In normal persons, this structure is usually larger in the dominant hemisphere. Abnormal architecture and cell clusters of the cerebral cortex (e.g., cortical dysplasia and ectopias) have been identified in these persons, suggesting that there may have been failure of normal neuronal migration or excessive neuronal death during fetal development.

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Michael D. Lumpkin

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1. INTRODUCTION

The term *neuroimmunology* means different things to different biomedical scientists and clinicians. This evolving and broad discipline includes the study of secreted immune cell products known as *cytokines*, *lymphokines*, *monokines*, and *chemokines* and their presence and actions in the central and peripheral nervous systems. Another area of study is that in which the *nerve supply and nervous system regulation of lymphoid organs* such as the spleen and thymus gland are considered. A third connotation of this word involves the investigation of how *neuroendocrine* substances of the hypothalamic-pituitary unit regulate the proliferation and activities of *monocytes*, *macrophages*, *lymphocytes*, and *glial cells* (considered by some to be the “macrophages of the brain”). A fourth implication of neuroimmunology is recognition of the fact that immune cells can produce neuroendocrine peptides such as adrenocorticotrophic hormone (ACTH) and luteinizing hormone releasing hormone (LHRH). A fifth important aspect of neuroimmunology involves the ability of the body to produce *antibodies* that can attack *cholinergic receptors* at the neuromuscular junction and the *myelin* components of the white matter of nervous tissue. A common theme that exists throughout all of these approaches to the understanding of neuroimmunology is that there is

bidirectional communication between the cells of the nervous system and the immune system. In some instances, neurons communicate with the immune cells (and vice versa) by way of endocrine messengers interposed between these other two systems. An example of this is when a stressful event causes the cytokine interleukin-1 (IL-1) to be produced by either activated monocytes, glial cells, or neurons residing in or traveling through the *hypothalamus* of the brain. The IL-1, in turn, causes certain hypothalamic neurons to release *corticotropin releasing factor (CRF)*, which then stimulates adrenocorticotrophic hormone (ACTH) secretion from the anterior pituitary gland. The ACTH next stimulates the production of *cortisol* by the adrenal gland, and this glucocorticoid then suppresses further proliferation and cytokine secretion by monocytes, lymphocytes, and glial cells. In addition, the cortisol also exerts a classic *negative feedback* action on both the CRF neurons and the pituitary corticotrope that manufactures ACTH in order to reduce their increased levels of secretion. Thus, the “*bidirectional communication*” occurs between these three great systems of the body (Fig. 1).

2. INNERVATION OF LYMPHOID ORGANS

Some of the original concepts about neuroimmunology have come from the work of Dr. David Felten. He and others have shown that *noradrenergic*

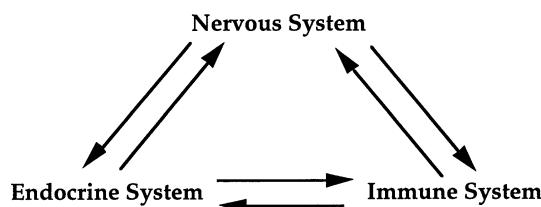


Fig. 1. Bidirectional communication occurs among components of the nervous, endocrine, and immune systems. Neuroimmunomodulation occurs throughout the organism by means of these interactions and feedback loops, and a stimulus to any one of these systems inevitably involves the others in the resultant reactions.

(NA) nerve fibers originating from the postganglionic sympathetic nervous system innervate the thymus gland, spleen, lymph nodes, and intestinal Peyer's patches. These norepinephrine-containing nerve fibers have been seen in contact with thymocytes, B lymphocytes, and macrophages. Further, both alpha and beta adrenergic receptors have been found on the surfaces of these immune cells. In general, activation of the sympathetic nervous system or administration of epinephrine produces leukocytosis, lymphopenia, and suppression of natural killer (NK) cell activity. Stated another way, activation of catecholaminergic beta receptors on lymphocytes inhibits their proliferation and results in a suppressed immune response. The role of catecholamines in suppressing lymphocyte activity has caused some to believe this is one explanation for why prolonged stress, which activates the sympathetic nervous system, leads to greater susceptibility to disease, infections, and cancer in human patients. For instance, it has been shown that the stress of foot shock, cold exposure, maternal deprivation, or fear may cause thymus gland involution, decreased lymphocyte proliferation and cytotoxicity, and diminished antibody production. Conversely, it was found that sympathetic denervation of immune organs causes proliferation of lymph node, spleen, and bone marrow cells, as well as antigen-stimulated immunoglobulin production. Interestingly, the use of mind-body medicine stress reduction techniques such as meditation, imagery, biofeedback, and breath control can also reduce sympathetic outflow from the CNS to the immune organs. These relaxation methods, when employed by stressed individuals such as medical students, have been shown to increase the number of CD4 T lymphocytes and NK cell activity, thus improving the students' immune status.

Dr. Felten's laboratory has also been instrumental in showing that a number of peptides found in autonomic nerves innervating the thymus, spleen, and lymph nodes are also regulators of cells of the immune system. In particular, *vasoactive intestinal peptide (VIP)*, *neuropeptide Y (NPY)*, and *substance P (SP)* have been observed in nerve fibers that make contact with thymocytes, lymphocytes, and macrophages found in the thymus gland and spleen.

Not unexpectedly, the receptors for these peptides have been described on lymphoid cells. The most studied of these nerve-ending peptides is VIP and its lymphoid receptor. When VIP is released from these nerve endings, it may act to inhibit the proliferation of lymphocytes, inhibit NK cell activity, and alter antibody production.

Some peptidergic nerve fibers that innervate lymphoid organs will produce the peptide substance P. In keeping with its known proinflammatory action and its role in mediating pain responses via peripheral nerves, activation of receptors by substance P stimulates lymphocyte proliferation and the release of pain mediators such as histamine and leukotrienes from mast cells. The peptide *somatostatin*, which is found both in the brain and peripheral nerves and which is mostly inhibitory to all types of cellular activity, can block the release of substance P from peripheral nerve endings.

The peptide NPY has been found in the same nerve fibers and terminals that contain norepinephrine and that innervate the thymus, spleen, and lymph nodes. It is likely that NPY and norepinephrine are co-secreted and act in synergistic fashion to suppress lymphocyte proliferation and cytokine release in keeping with the general idea of sympathetic nervous system inhibition of immune function.

Another example of the commonality between the brain and the immune system is the fact that other neuropeptides and their receptors are found in both locations. This would include CRF, ACTH, enkephalins, beta-endorphin, vasopressin, oxytocin, and neuropeptides. It is useful to note that most of these neuropeptides are also released during episodes of physical or psychological stress. Further, these peptides are mostly inhibitory to immune cell proliferation and function. Thus the suggestion has been made that these peptides may mediate the suppression of the immune system and the subsequent susceptibility to disease and infection that often follows periods of prolonged stress in an individual.

3. CYTOKINES IN THE NERVOUS SYSTEM

It is readily known that immune system cells produce and secrete soluble mediators known as cytokines whose original defined role was to modify the proliferation and activity of other cells of the immune system. However, it is now appreciated that activated immune cells including monocytes, lymphocytes, and macrophages can breach the blood-brain barrier and establish themselves as a presence in the brain where they may release their specific cytokines into the cerebrovasculature, the cerebrospinal fluid, and the parenchyma of the CNS. In addition, microglial cells and, to a lesser extent, astroglial cells of the brain secrete the cytokines interleukin-1, 2, 4, and 6 and tumor necrosis factor alpha (TNF-alpha). Accordingly, the receptors for IL-1, 2, and 6 have also been identified in the brain. Most provocative is the recent finding that IL-1 is also produced by neurons, particularly in the hypothalamic and hippocampal neuronal populations that are involved in stress and homeostatic responses. Likewise, IL-1 receptors have also been identified in the hypothalamus and hippocampus.

Physical stressors such as trauma, infection, and inflammation of brain tissue as well as psychological stressors stimulate the production of cytokines such as IL-1 by glial cells and neurons. IL-1, IL-6, and TNF, when so manufactured by brain cells for prolonged periods in pathologic states, can produce changes in neural functions such as anorexia, fever, sleep induction, dementia, and even neuronal death. During invasion of the brain by the human immunodeficiency virus (HIV) and in patients with Alzheimer's disease, the levels of IL-1 are increased and contribute to the behavioral disturbances observed in these patients. However, the brain has apparently developed a protective mechanism against the persistent presence of pathologically elevated IL-1 levels as such situations also lead to the subsequent production of an *endogenous IL-1 receptor antagonist* that is homologous to the structure of IL-1 and competes for the same receptor in order to dampen further IL-1 activity. IL-1 receptor antagonist has no known inherent activity of its own except to occupy the IL-1 receptor and block its activation by IL-1.

Whereas sustained high levels of cytokines in the brain produce deleterious effects on neuronal function, normal physiologic oscillations in brain cytokines in response to acute changes in homeostatic activity or intermittent stressors are beneficial to the organism. The presence of physiologic concentrations of IL-1, IL-2, and IL-6 in the hypothalamus

appears to regulate certain neuroendocrine axes. For instance, IL-1 produced in or injected into the brains of experimental animals increases the expression of CRF mRNA and synthesis and release of its neuropeptide product. This in turn causes the secretion of ACTH from the anterior pituitary gland. The ACTH then binds to its receptors on the cortex of the adrenal gland leading to the increased secretion of glucocorticoids such as cortisol. This corticosteroid provides a classic negative feedback signal back to the neurons, glia, and monocytes/macrophages that provided the elevated levels of CRF-stimulating cytokines in the first place. Elevated cortisol levels then decrease both the secretion of cytokines from glial cells and neurons as well as reduce the proliferation and secretion of cytokines from monocytes and lymphocytes. More specifically, cortisol will inhibit the production of IL-1, IL-2, IL-6 receptors, interferon-gamma, and immunoglobulin production. In this fashion, the increased activities of immune cells and neuroendocrine cells in response to physical or emotional stressors are prevented from running amok. This is important during a stress-induced cortisol release period where it may be necessary to suppress inflammatory responses during a "fight or flight" situation. Stated differently, cortisol downregulates IL-1, IL-2, IL-6, and CRF secretion thereby maintaining homeostatic concentrations of cytokines and hormones in the body, specifically in response to acute stress.

Another beneficial effect of IL-1 may occur after nervous tissue injury. This positive influence derives from IL-1's ability to stimulate the synthesis and secretion of nerve growth factor (NGF). NGF acts as a neurotrophic growth factor that promotes both the healing and the regeneration of certain types of nerve cells.

IL-1 and its related cytokines also exert effects on other neuropeptides of the brain. IL-1 can act both directly on other releasing factor systems and through the stimulation of CRF, the neurons of which make synaptic contact with growth hormone-releasing hormone (GHRH), luteinizing hormone-releasing hormone (LHRH), and thyrotropin-releasing hormone (TRH) nerve cells. In each case, the master stress hormone CRF, when activated, will inhibit these other neuroendocrine axes in addition to stimulating adrenal cortical production of glucocorticoids. In the short-term, the organism benefits from increasing cortisol levels. The cortisol will ensure that blood glucose concentrations are maintained for the increased stress-induced demands on the brain,

heart, and skeletal muscle; for enhancing catecholamine production by the adrenal medulla; and for sustaining the blood pressure and blood flow to critical tissues. In other words, the stress hormone system is turned on and other hormonal systems for growth, reproduction, and metabolism are turned off to spare resources for the stress response. Further indicative of the integrated nature of the stress response, stimulation of CRF elements in the hypothalamus not only produce ACTH and cortisol release but also stimulate sympathetic outflow from the brain to visceral and immune organs. This would further suppress immune system activity. IL-1 alone and/or through CRF neuronal mediation acts in the hypothalamus to increase somatostatin synthesis and secretion, while in a coordinated fashion it also inhibits the release of GHRH. The overall consequence of this action is to reduce growth hormone secretion from the anterior pituitary. Speculation exists that this mechanism may contribute to the protein wasting syndrome in adult patients with HIV infection of the brain and to the failure of somatic growth in children suffering from acquired immune deficiency syndrome (AIDS). The reason for this, of course, is that growth hormone is anabolic for protein synthesis of muscle and connective tissue and stimulates the growth of both long bones and soft tissues in children. Thus an IL-1-induced GH deficiency could account for the failure to thrive and negative nitrogen balance characteristic of AIDS patients.

IL-1 in the hypothalamus also inhibits the secretion of LHRH and TRH. It is recognized that sustained increases in IL-1 that might be encountered during prolonged stressful situations such as chronic viral infections can lead to amenorrhea in women and decreased spermatogenesis accompanied by lowered testosterone levels in men, the outcomes being decreased libido and reproductive failure. Hormonally, this deficit is seen as the cessation of the normal pulsatile secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary gland. Without sufficient stimulation of the gonads by LH and FSH, both sex steroid synthesis and germ cell production will fail.

Consistent with their many inhibitory activities on various brain functions, both IL-1 and TNF-alpha suppress thyroid function directly at the level of the thyroid gland but also via the inhibition of TRH production by the hypothalamus. Incidents of inflammation and sepsis decrease thyroid stimulating hormone (TSH) secretion from the pituitary because IL-1 from both peripheral and central sources inhibits TRH

secretion, and TNF-alpha lowers the hypothalamic mRNA content of TRH. These actions result in lowered plasma thyroid hormone levels leading to a reduced metabolic rate, perhaps aggravating the fatigue and lethargy caused by the action of IL-1 and TNF directly on the brain, particularly in AIDS patients.

Currently in therapeutic development are specific IL-1 and CRF receptor antagonists that could be administered to patients with disorders related to chronic stress. Such therapeutic agents would have the potential to enhance immune function in chronically ill patients by relieving CRF- and cortisol-induced suppression of immune cells and other immunostimulating hormones such as growth hormone and thyroid stimulating hormone.

4. PRESENCE OF NEUROENDOCRINE PEPTIDES IN IMMUNE CELLS

Another recent development in the field of neuroimmunology is the discovery by Dr. Edwin Blalock and his colleagues that neuroendocrine peptides are produced by immunocompetent cells. For instance, B lymphocytes contain ACTH and enkephalins, the secretion of which can be stimulated by CRF and inhibited by glucocorticoids. The T cells synthesize growth hormone, TSH, LH, and FSH. Monocytes are a source of prolactin, VIP, and somatostatin. As a general rule, when a "pituitary" hormone is found in a cell of the immune system, the corresponding releasing factor peptide is also found there and usually has the ability to stimulate the secretion of its "target" hormone from a particular type of immune cell. An example of this is the presence of GHRH in lymphocytes, which may be secreted *locally* among other lymphocytes and act on GHRH receptors on the lymphocyte cell membrane, thereby eliciting the *local* secretion of growth hormone by like cell types. The same is true for TRH/TSH and LHRH/LH, which have been located in immune cells. However, such mechanisms of local control are controversial and not well-studied at this time. It is important to note that while the secretion of neuropeptides by monocytes and lymphocytes may provide significant levels of *paracrine* and *autocrine* regulation among immune cells, there is little evidence to suggest that lymphocyte-derived releasing factor peptides or pituitary hormones exert actions on peripheral endocrine glands. The extremely small quantities of neuropeptides secreted by lymphocytes essentially preclude them from playing major roles as circulating hormones with peripheral endocrine glands as their targets.

5. NEUROIMMUNOMODULATION BY NEUROENDOCRINE PEPTIDES

Having established the presence of various neuroendocrine peptides in and among the immune cell population, it is useful to understand how they affect the immunoregulatory activities of these cells. In the inhibitory category, ACTH suppresses macrophage activation and the synthesis of antibodies by B cells. Gonadotropins decrease the activity of T cells and natural killer cells. Somatostatin and VIP inhibit T-cell proliferation and the inflammatory cascade. These actions are somewhat opposed by growth hormone and prolactin (which are homologous in their structures) as these peptides can stimulate lymphocyte proliferation, antibody synthesis, and oppose the inhibitory effects of glucocorticoids on lymphocyte proliferation and cytokine production. Further, growth hormone and prolactin stimulate the growth and activity of the thymus gland and lymphoid cells. Growth hormone also functions as a macrophage activating factor, and prolactin enhances the tumoricidal activity of macrophages and the synthesis of interferon-gamma. TSH enhances antibody synthesis, and beta-endorphin stimulates the activity of T, B, and NK cells.

6. AUTOIMMUNITY AND NEUROIMMUNOLOGY

Perhaps the best example of autoimmunity in human neurologic disorders is that of myasthenia gravis in which autoantibodies attack acetylcholine (ACh) receptors, rendering them unresponsive to the ACh secreted into the synaptic cleft. Two mechanisms involved in this loss of receptor responsiveness include complement-mediated lysis and downregulation of the ACh receptor secondary to cross-linking of receptors. The precipitating event in the origin of myasthenia gravis is not understood. However, it is possible that T cells of the thymus gland may mediate this type of autoimmune response. The following chapter discusses this autoimmune disorder in greater detail, and the reader is referred there for more information.

Another example of derangements in normal neuroimmunologic function is that represented by the chronic inflammatory neural disease multiple sclerosis (MS). In this disease, there is demyelination of CNS neurons with the subsequent loss of neuronal function. T-cell-mediated immunity is the principal mechanism in which autoimmunization to myelin

antigens results from actual immunization with myelin components or infection with cross-reactive viruses or viral proteins. Antibodies to myelin basic protein and other myelin breakdown products appear in CSF and plasma. In addition, this condition results in an elevation of other nonantibody products in blood and CSF. The activation of lymphocytes and macrophages in MS increases levels of interferon-gamma and prostaglandin E₂. Interestingly and consistent with the previous information in this chapter, the activated immune cells in MS patients secrete elevated levels of ACTH, prolactin, beta-endorphin, and substance P. At this time, however, the roles of these peptides and hormones on the clinical state of the MS patient is unknown.

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Sonia L. Carlson Watson and Dwight M. Nance

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1. INTRODUCTION

It was once believed that the nervous system and immune system were separate and independent systems, each with their own regulatory systems. However, it is now clear that these systems overlap and communicate extensively on a continuous basis, both as a part of normal function and in disease and trauma. It has been clearly shown that these two systems share common receptors and ligands for hormones, neurotransmitters, and cytokines, which forms the basis for this communication. In addition, as discussed here, the central nervous system (CNS) is not exclusively “immunoprivileged,” although it does have the capacity to limit the activation of an inflammatory response under many circumstances.

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2. INTERACTIONS OF THE NERVOUS AND IMMUNE SYSTEMS IN THE PERIPHERY

2.1. Innervation of Lymphoid Tissue

Peripheral lymphoid tissues receive extensive innervation by nerve fibers, particularly from the sympathetic nervous system. Prominent innervation by neuropeptides has also been documented. All lymphoid tissues, including primary (bone marrow, thymus) as well as secondary lymphoid tissues (spleen, lymph nodes, and gut-associated lymphoid tissue), have been found to be innervated by nervous system fibers. Innervation of lymphoid tissues by the sympathetic nervous system has been the most extensively characterized. In tissues such as spleen, this innervation is found as a plexus surrounding the vasculature, entering with the splenic artery and continuing in a subcapsular plexus. Fibers also extend into the trabeculae and enter the white-pulp areas in association with the central arterioles, where branches extend

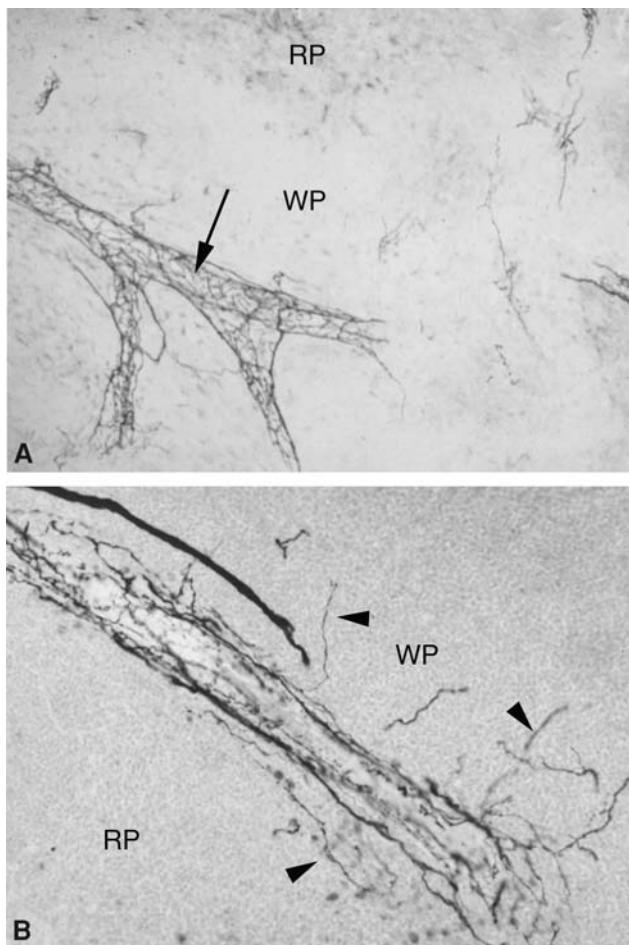


Fig. 1. Sympathetic innervation of the murine spleen. Sympathetic nervous system fibers were revealed using an antibody against tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of catecholamines. **(A)** Robust innervation is found surrounding the central artery (arrow) of the white pulp (WP), an area heavily populated with T cells and with B-cell nodules. Little innervation is found in the red pulp (RP). **(B)** A higher-magnification view of an area of murine spleen shows that individual nerve fibers (arrow heads) can be found in the midst of lymphocytes in the periarteriolar lymphatic sheath (PALS) of the white pulp. This region is heavily populated with T cells; B-cell nodules have little innervation. The tissue has been counterstained with methyl green, thus, the lymphocyte nuclei can be faintly seen in the white pulp.

into the parenchyma in close proximity with lymphocytes (Fig. 1). In general, this innervation is most prominent in T-cell zones of the tissues (periarteriolar lymphatic sheath) and in the marginal zone that is heavily populated by macrophages. Much more sparse innervation is found in B-cell zones and follicles. A similar pattern is found in lymph nodes where the subcapsular and medullary regions are innervated, with fibers extending into the T-cell zones of the cortex. Little innervation is found in the B-cell

follicles. In the medulla, the nerve fibers are found in association with both vascular and lymphatic channels. Innervation has also been documented in other lymphoid tissues such as the bone marrow, thymus, and gut-associated lymphoid tissues, which is discussed extensively in the suggested readings.

Neuropeptides have also been found to innervate the primary and secondary lymphoid tissues. These include neuropeptide Y (NPY), substance P, calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), somatostatin (SS), and opiate peptides. Peptides such as NPY often appear to be colocalized with the noradrenergic sympathetic innervation. VIP is found in similar compartments but not colocalized with the noradrenergic fibers. The neuropeptides substance P and CGRP are often colocalized; they can be found along the vasculature but also extend into the parenchyma. Their distribution is somewhat different than the sympathetic innervation. Although substance P and CGRP are generally associated with sensory systems, detailed tract tracing studies have found no evidence for sensory input (vagal or dorsal root ganglia) to the thymus and spleen. However, there is some evidence of a sensory input to regional lymph nodes that may fit with the role of sensory input to the adjacent tissues in regulating local inflammatory responses.

2.1.1. NEURAL INNERVATION MODULATES IMMUNE FUNCTION

In order for the innervation of lymphoid tissues to modulate immune function, the cells of the immune system must express receptors for the neurotransmitters. Indeed, receptors for catecholamines as well as numerous neuropeptides have been proved to be present on the various subsets of leukocytes. Thus, cells of the immune system are capable of responding to stimulation by neurotransmitters released by nerves that innervate lymphoid tissues. The catecholamine receptors have been most extensively characterized, with the predominant receptor expressed being the β_2 -adrenergic receptor subtype. Stimulation of this receptor causes production of intracellular signals, namely increased levels cAMP and subsequent activation of protein kinase A (PKA). It also can stimulate other intermediates such as mitogen-activated protein kinase (MAPK). Denervation and agonist/antagonist treatment paradigms have shown that modulating the level of catecholamines and neuropeptides can significantly alter a variety of responses from the immune system, including cytokine production, lymphocyte proliferation, antibody production,

and cytotoxic functions. In addition to direct intracellular effects, catecholamines and neuropeptides have been shown to modulate the vasculature of lymphoid tissues by affecting vasodilation and adhesion-molecule expression. This could affect leukocyte trafficking to lymphoid tissues and sites of inflammation as well as leukocyte retention within the lymphoid tissues. Thus, there are numerous actions through which neural innervation can modulate localized inflammatory responses.

It is difficult to generalize about the functional role of lymphoid tissue innervation because interpretation of the literature is complicated by the fact that the response found is affected by the combination of cell types included in the analysis and the time-points examined. Particularly related to the sympathetic nervous system, sympathetic activation (and release of norepinephrine) tends to result in inhibition of cell activity related to the innate immune system. The innate immune system includes the phagocytes (macrophages and neutrophils), natural killer cells, and granulocytes. For example, norepinephrine is a potent inhibitor of macrophage tumor necrosis factor- α (TNF- α) production. Activation of sympathetics can either enhance or inhibit immune cell activity related to the acquired/adaptive immune system (the arm of the immune system that includes antigen-specific T and B cells). Because the activity of macrophages is integral to the activation and regulation of T and B cells, neural input that affects the innate immune response can also have an effect on the magnitude and quality of the subsequent adaptive immune response. Overall, the interaction of the nervous and immune systems should probably be viewed as one in which the neural input modulates the extent of an inflammatory response as opposed to controlling or regulating it. Under normal circumstances, it may provide some of the feedback that prevents overstimulation of the immune response so that the response remains specific and is terminated at the appropriate time. The innervation of lymphoid tissue is also important in mediating the effects of the CNS on peripheral immune function and is an important factor in the effect of chronic stress on immune function.

2.2. Neuroendocrine Interactions with the Peripheral Immune System

In addition to direct neural input to lymphoid tissues, the CNS can modulate immune function through outflow from the neuroendocrine system. The effect of the hypothalamo-pituitary-adrenal axis

is especially well documented. In this axis, physiologic stimuli such as stress can rapidly signal an increase in adrenocorticotrophic hormone (ACTH) and glucocorticoid production, which in turn can stimulate cells of the immune system that possess glucocorticoid receptors. It was originally believed that stress was inhibitory to immune function because glucocorticoids can be immunosuppressive. Several studies have shown that stress is not universally inhibitory, as some levels of stress have been found to positively affect certain measures of immune function. Prolonged or repeated extreme or inescapable stress, however, has been shown to be associated with reduced immune responses. The effect of stress is not mediated entirely through effects on glucocorticoids but also through the outflow from the sympathetic nervous system, which modulates activity of the sympathetic innervation of lymphoid tissues and the release of epinephrine from the adrenals. A review of the literature may allow a few generalizations to be made concerning the effects of stress on lymphocyte distribution and function: acute stress, which is particularly associated with the rapid release of catecholamines, can stimulate a rapid (within minutes) release of leukocytes into the general circulation. As glucocorticoid levels are increased over the next few hours, the increase in leukocytes is reversed as the leukocytes redistribute to tissues such as skin and lymph nodes, resulting in a decrease in circulating leukocytes. The number of leukocytes in the blood is not a direct indicator of immune function, however, as specific immune responses are occurring at localized tissue sites. In terms of immune function, acute stress has often been shown to inhibit innate immunity (i.e., macrophage production of TNF- α) and enhance humoral and cell-mediated immunity, whereas chronic or repeated severe stress can result in immunosuppression. Thus numerous factors determine the ultimate outcome of stress on immunity. In addition to the ACTH-glucocorticoid axis, many other hormones of the neuroendocrine system have been shown to interact with cells of the immune system, including growth hormone, thyroid hormone, β -endorphin, leuteinizing hormone-releasing hormone (LHRH), prolactin, SS, corticotrophin-releasing hormone (CRH), and others. Receptors for numerous neuroendocrine hormones have been found on leukocytes, and there is some evidence of neuroendocrine hormone production by leukocytes, although the levels are generally very low.

In summary, there are two major outflow systems from the CNS that can communicate with and

modulate responses from the immune system: the neuroendocrine system and the peripheral outflow through the sympathetic nervous system. There is extensive evidence that these systems are in constant communication and act together in normal homeostasis. However, extreme perturbations can be associated with maladaptive responses and compromised immune function.

2.3. Immune Signaling to the CNS

In contrast with CNS outflow that communicates with and modulates the peripheral immune system, products of the immune system can signal the CNS in several ways. Presumably, this is to alert the CNS to inflammatory events occurring in the periphery as part of a system to maintain homeostasis or to coordinate a CNS response to the immune activation. It has been long recognized that peripheral activation of the immune system results in a host of “sickness behaviors” that are initiated at the level of the CNS. These sickness behaviors include induction of fever, increased sleep (particularly slow-wave sleep, or SWS), hyperalgesia, and stimulation of the ACTH-glucocorticoid axis and of the sympathetic nervous system. One of the most important cytokines in mediating these effects is interleukin 1 (IL-1). Cytokines such as IL-1 produced in the periphery can signal the CNS through different pathways. The first means is through direct stimulation of the CNS from blood-borne cytokines. Cytokines are too large to enter the CNS through passive diffusion; however, there is evidence for active transport of cytokines across the blood-brain barrier, although the levels transported by this means are relatively small. The transport can occur at endothelial cells of the brain and spinal cord and the choroid plexus. Cytokines shown to have a saturable transport into the CNS include IL-1, IL-6, and TNF- α . Another route of communication is at the circumventricular organs (CVOs), areas of the brain that lack a blood-brain barrier. The uptake of cytokines is facilitated through transport mechanisms, so that levels of cytokine are elevated over what is found at other CNS sites. Cytokines at CVOs do not directly diffuse into deeper CNS structures, however, because of the cells at the CVO-brain interface. Subsequent signaling into the CNS is in part through the production of prostaglandins in response to the cytokine. Prostaglandins are lipophilic and thus can diffuse into the CNS parenchyma and stimulate neurons or induce cytokine production by various cells within the CNS. This, in turn, can

stimulate endocrine and autonomic centers to initiate the sickness response.

Another pathway for peripheral cytokines to stimulate the CNS is through vagal nerve afferents that synapse in the nucleus solitarius and the area postrema (AP), areas that are the first to become activated after peripheral administration of IL-1 or lipopolysaccharide (LPS). LPS is derived from the cell walls of Gram-negative bacteria and is a potent stimulator of macrophages. This mechanism is most likely to be important in signaling the brain in response to localized inflammation in tissues innervated by vagal afferents, including the lung and viscera of the gut. It has been shown that cutting the vagus nerve at a subdiaphragmatic level results in loss of many of the sickness behaviors and responses in the hypothalamus and preoptic area that are initiated after injection of IL-1 or LPS into the gut. Many details of the signaling via this route still must be established; however, there is evidence that locally produced IL-1 can either directly stimulate vagal afferents or the paraganglion cells that could subsequently stimulate the vagus. Likewise, sensory nerves located elsewhere in the body, such as skin and muscle, can also provide sensory information to the CNS from sites of localized inflammation and injury.

In conclusion, there appear to be two major routes for peripheral cytokines to stimulate the CNS that can initiate sickness behaviors. First, there is evidence for direct signaling of the CNS at the level of the vasculature or at the CVOs that is from blood-borne cytokines. Second, localized production of cytokines in the abdominal or thoracic viscera, skin, and muscle can alert the CNS to an infection through vagal and musculocutaneous afferents. Thus, there is communication to the CNS in response to infection or tissue injury in the periphery that allows a coordinated response to deal with the inflammation.

3. IMMUNE INTERACTIONS WITHIN THE CNS

Although it is commonly believed that the CNS is an immune-privileged site, it is now apparent that this is more likely the result of a relative lack of the immune-related molecules and costimulatory signals that would allow normal immune responses to occur within the CNS, as opposed to a complete lack of leukocyte trafficking through the CNS. The existence of tight junctions between endothelial cells in the capillaries of the brain does highly regulate which molecules cross the blood-brain barrier, thus limiting

access of cells and molecules to the CNS. Another means of protection is the tissue environment that exists within the CNS, which tends to prevent the activation of an immune response. As part of the regulatory system that helps to control and direct immune responses, the cells of the immune system cannot be stimulated in isolation, but instead require a series of cellular interactions and the development of a cascade of events to allow a full immune response to occur. Among the molecules that are expressed by all cells that help to identify self are the major histocompatibility complex (MHC) antigens, in particular, MHC class I. Expression of this molecule is extremely limited within the CNS under normal circumstances. Foreign antigens are recognized by cells of the immune system in association with MHC molecules, which in turn signals the immune system to initiate a response. The lack of MHC expression blocks some of the basic signaling that is required to initiate the process of an immune response. In addition, cells such as the microglia of the CNS normally express limited amounts of MHC class II that is also important in antigen presentation and initiation of inflammatory responses. It is likely that the nervous system developed a means to protect itself from spurious inflammatory responses and “remodeling” resulting from inflammation or phagocytosis, which would have harmful effects on a system that must maintain consistent connections. However, this protection is at the expense of repair mechanisms that exist in the periphery.

Another method that limits inflammatory responses to antigens within the CNS is the relative lack of lymphatic drainage from the CNS to lymph nodes. In peripheral tissues, the generation of an effective immune response is partly caused by the drainage of antigens to the draining lymph nodes, where interactions are optimized to allow the appropriate cells to interact and initiate a full response. In the CNS, there are no classic lymphatic channels to drain directly into regional lymph nodes. However, antigens can ultimately reach these lymph nodes through the drainage of the interstitial fluid of the brain into the cerebrospinal fluid (CSF), which subsequently drains into the venous system at the arachnoid villi. In addition, some CSF drains along some of the cranial nerves and spinal nerve roots to lymphatics, thus providing a more direct method for proteins from the CNS to reach regional lymph nodes. There is evidence to suggest that this can result in the generation of a noninflammatory humoral immune response.

Additional evidence for the relative protection of inflammatory responses within the CNS comes from studies that involve transplantation of cells or tissue into the CNS. Even with minor MHC incompatibility, the transplants are often not rejected. However, if the peripheral immune system is exposed to the same tissue, an inflammatory response is generated that results in the production of cytotoxic T cells. These activated cells are subsequently able to cross the intact blood-brain barrier and stimulate rejection of the transplant. Thus, activated T cells are able to migrate into the CNS. It is likely that there is a continuous surveillance of the CNS by cells such as activated T cells, but under normal circumstances there are few stimuli within the CNS to further activate these cells or to promote the generation of an inflammatory response.

3.1. Microglia Within the CNS

Microglia are considered by many to be the macrophages of the CNS. Microglia are widely dispersed throughout the brain and spinal cord and are present in both the gray matter and white matter. Microglia are normally highly ramified, with a relatively small cell body and highly branched, thin cellular processes. Under normal conditions, microglia appear to be resting cells with a relatively small amount of perinuclear cytoplasm or organelles. The morphology of microglia is affected by the local microenvironment in various regions of the CNS, and the morphology can be strikingly different. Microglia within gray and white matter have a ramified morphology, although the pattern of the processes can be distinct in different regions. Microglia in the gray matter have processes that are ramified in all directions, whereas white-matter microglia often have processes that are arranged in a more linear fashion (Fig. 2A). In contrast, microglia in the CVOs are more compact with short processes, suggesting a more activated morphology in these regions that lack a blood-brain barrier. There also are regional differences in microglial density, with high densities present within areas such as the substantia nigra, ventral pallidum, and olfactory tubercle. Microglia show very slow turnover, with a small amount of proliferation within the normal CNS. Through the use of cellular labeling and chimera studies, there also is evidence for small amounts of renewal through migration of macrophages into the CNS, although this is a slow process. Although there is little proliferation of microglia within the normal CNS, the capacity to proliferate is increased after injury or trauma.

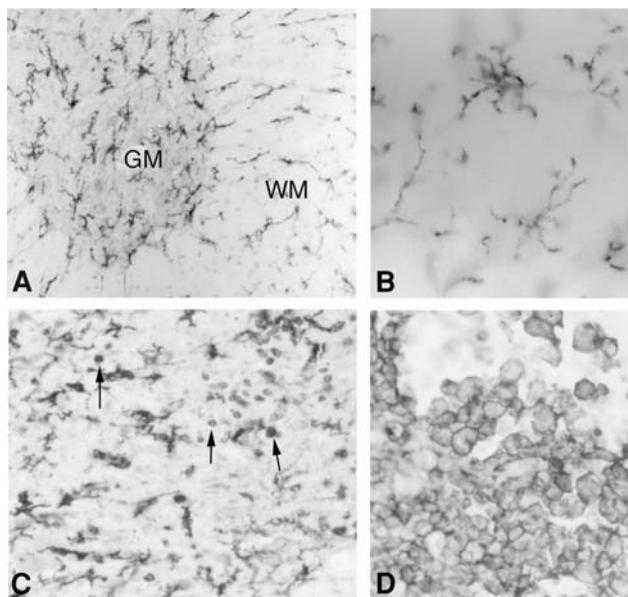


Fig. 2. Morphology of microglia and macrophages in the spinal cord. Microglia of the rat spinal cord were visualized with the OX-42 antibody that recognizes the C3b complement receptor. (A) Microglia of the gray matter (GM) have a more stellate pattern compared with the more linear pattern of microglia of the white matter (WM). (B) A higher-magnification view of normal gray-matter microglia reveals that they have numerous fine processes extending from the cell body. (C) Microglia become activated after traumatic injury to the spinal cord. This view taken 24 h postinjury shows that many microglia have become activated and have begun to retract their processes so that they appear thicker and more blunt. Some microglia or macrophages have a round phagocytic morphology (arrows). (D) One week postinjury, the macrophages/microglia have engulfed much tissue debris and have become greatly enlarged.

In addition to the resident microglia within the CNS, macrophages are also found in the perivascular spaces in the CNS. These cells lack the highly ramified morphology of the microglia and are found to be associated with the vasculature and the basement membrane that surrounds the CNS vessels. These cells show greater turnover than microglia, as new macrophages from the blood can enter this space. Macrophages are also normally found in other areas of the CNS including the choroid plexus, the leptomeninges, and on the walls of the cerebral ventricles.

3.2. Function of CNS Microglia and Macrophages

As stated previously, cells of the CNS express very low levels of molecules that normally would be required to initiate an immune response. In keeping with this, resting microglia express little or no MHC

class I and few express MHC class II. Thus, they do not appear to be acting as antigen-presenting cells on a continuous basis. It is not clear whether resting microglia are playing a functional role during times of normal homeostasis within the tissue. However, microglia are extremely sensitive to perturbations in the local environment and thus can respond rapidly to toxic stimuli or to trauma. Microglia can rapidly change from the resting state, where they exhibit the morphology noted previously with an extensive arbor of fine processes, to an activated state that can take on a range of forms (Fig. 2C, D). The initial response involves upregulation of a number of cell-surface markers, including the MHC molecules, complement receptors, CD4, and others. In addition, the morphology of the microglia changes as the fine processes are retracted, resulting in a more hypertrophied cell body and thicker, more blunt processes (Fig. 2C). With sufficient activating stimuli, microglia can become fully activated and achieve what is often referred to as a phagocytic morphology, in which the cells become rounded and cannot be distinguished from peripheral macrophages (Fig. 2C, D). Indeed, in areas of trauma and breakdown of the blood-brain barrier, it is not possible to fully discern which cells are activated microglia versus macrophages that have entered the tissue from the vasculature. The nature of the stimulus affects the macrophage response that is elicited. Differences in the magnitude and timing of the response can differ, depending on whether the scenario includes an intact versus a breached blood-brain barrier, and if the neurons or glia in the area are undergoing cell death.

There is much debate as to whether the activation and recruitment of microglia and macrophages to sites of injury are beneficial or contribute to further damage. Clearance of tissue debris after injury or myelin from areas undergoing Wallerian degeneration is crucial for the process of repair and regeneration. There is much evidence, however, that the rate of macrophage recruitment into these sites is slower than what occurs in the periphery, and the functional ability of macrophages and microglia to phagocytose and remove the debris is considerably slower within the CNS. Thus, there appear to be inhibitory molecules present in the CNS that reduce these aspects of macrophage/microglial function that is ultimately detrimental to regeneration. Myelin contains molecules that are inhibitory to axonal regeneration; thus, prolonged presence of myelin and other tissue debris is believed to underlie part of the relative inability of the CNS to recover from injury to the same extent as is found in peripheral nerves.

In contrast, the chronic activation of microglia is believed to underlie some of the damage that can occur in the CNS during certain neurodegenerative diseases. Microglia are capable of producing numerous cytokines, including IL-1 and TNF- α , which are associated with promoting inflammation. These cytokines can act on endothelial cells to induce edema and leakiness of the blood-brain barrier, thus allowing greater access of cells and molecules into the CNS that normally would be tightly regulated. These cytokines also can induce the expression of adhesion molecules that promote the influx of inflammatory cells and promote the upregulated expression of molecules such as MHC and complement receptors that are used in promoting an inflammatory response. In conjunction with this, proinflammatory cytokines can induce the activation of macrophages and microglia so that they can release damaging mediators such as reactive oxygen species (ROS), nitric oxide, and damaging enzymes. Microglia can also be a source of potent proinflammatory bioactive lipids such as platelet-activating factor (PAF) that have many actions that are similar to the proinflammatory cytokines. In addition, PAF has been shown to be toxic to neurons and may be one of the molecules released by activated macrophages and microglia during diseases such as AIDS dementia that cause damage to neurons.

One interpretation regarding microglia of the CNS is that the role they play is dependent on the cellular cues present in the particular microenvironment and on the extent to which they become activated. Much experimental evidence suggests that microglia can have a positive role in promoting wound healing and regeneration through the release of trophic factors when they are activated, but not to the extent that they have achieved the phagocytic morphology. In this latter state, they are capable of producing an oxidative burst and of releasing degradative enzymes that can contribute to tissue damage. It is also possible that endogenous microglia may play a different role than do peripheral macrophages that are recruited into a site of damage. A better understanding of the positive and negative effects of these cells awaits a more complete characterization of the products produced by these cells in various activation states and in the context of different pathologies within the CNS.

3.3. Other Cells Within the CNS That Can Participate in Inflammatory Responses

Microglia are not the only cells of the CNS that can be part of an inflammatory cascade. Cells such as the

endothelial cells and astrocytes also play an important role. Endothelial cells provide much more than a barrier function between the blood and the brain. They are responsive to substances in the blood and to injury or trauma so that they can release proinflammatory substances (cytokines and bioactive lipids such as PAF and prostaglandins). These substances in turn can upregulate adhesion molecules (e.g., intercellular adhesion molecule 1 (ICAM-1), integrins, and selectins) expression by endothelial cells, which helps direct migration of leukocytes into a site of injury or inflammation. Proinflammatory molecules also promote alterations in the tight junctions between endothelial cells after injury or during an inflammatory response resulting in edema and more ready access of large molecules and inflammatory cells into the CNS. Endothelial cells, along with microglia, astrocytes, and other cells, can also release chemokines that act to actively direct leukocytes to follow the chemokine gradient into the tissue. Thus, endothelial cells play a dynamic role in the cascade of events that allows immune responses in the CNS to develop.

Astrocytes have been shown to produce cytokines and to respond to cytokines in the environment. Astrocytes have been shown to secrete numerous cytokines, including IL-1, IL-6, IL-8, TNF- α , IFN- γ , and TGF- β , among others. In turn, they can respond to many of these same cytokines, suggesting a possible autocrine loop. Cytokine activation of astrocytes can lead to a variety of responses, including effects on astrocyte growth and maturation, release of nitric oxide, and further production of cytokines. Astrocytes can also be induced to express adhesion molecules and MHC molecules, thus providing other means to participate in inflammation. The expression of MHC molecules by astrocytes and endothelial cells allows these cells to act in antigen presentation to T cells, although they are unlikely to be as important as microglia for this function. Cytokine stimulation has also been linked to production of nerve-growth factor (NGF) by astrocytes. The physical apposition of astrocytic end feet to ependymal cells and to capillaries allows a role in regulation of the entry of substances or cells into the CNS. Thus, astrocytes are now known to be involved in a wide range of functions, some of which interact directly with the immune system.

4. INFLAMMATORY RESPONSES IN THE CNS DURING TRAUMA OR DISEASE

The generation of an inflammatory response, including the recruitment of leukocytes, is dependent on the type of injury. Degeneration of a nucleus

within the CNS in response to peripheral axotomy may result in local activation of microglia but may not induce the recruitment of other leukocytes, such as neutrophils or peripheral macrophages. In direct trauma to the CNS, however, a robust inflammatory response is elicited that involves a full cascade of events that is not unlike what occurs in the periphery. This includes edema, rapid production of cytokines (particularly TNF, IL-1, and IL-6), upregulation of adhesion molecules on endothelial cells, and production of chemotactic agents. The chemotactic agents, or chemokines, help to direct which leukocytes enter and in what sequence because there are different classes of chemokines that are specific for neutrophils, macrophages, and lymphocytes. The timing of chemokine production generally correlates well with the influx of different subsets of leukocytes into the injured tissue. Although there is debate on the positive versus the negative effects of leukocyte influx into the damaged CNS, there is considerable evidence that the early, robust influx of neutrophils and macrophages after traumatic injury can contribute to secondary-injury mechanisms that expand the lesion beyond the original injury site.

The first leukocytes to enter the CNS after ischemia, traumatic cortical damage, or spinal cord injury are neutrophils. These cells represent the first-line, nonspecific arm of the immune system that enters the tissue rapidly, with a peak from 4 to 24 h after injury. Ischemia has been shown to cause a rapid influx of neutrophils into CNS tissue; thus, the amount of ischemia may modulate the rate of the neutrophil response. Neutrophil accumulation in the tissue is even greater in ischemia-reperfusion in which blood flow is reestablished because the blood supply can bring new neutrophils to the damaged tissue. This in turn is associated with even greater tissue damage. Neutrophil and other leukocyte influx is specifically directed into inflammatory sites by proinflammatory mediators produced by the damaged tissue. These mediators include cytokines such as IL-1, TNF- α , and bioactive lipids, including PAF and LTB4. These mediators stimulate a rapid increase in the expression of adhesion molecules on endothelial cells of postcapillary venules, which are then recognized by their counter-receptors on neutrophils. Selectin adhesion molecules mediate the initial adhesion of neutrophils in the blood to endothelial cells. Subsequent higher-affinity interactions of neutrophil integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) with endothelial-cell ICAM-1 are essential for neutrophil transmigration

across the endothelial barrier to enter the inflamed tissue. Endothelial ICAM-1 expression has been shown to increase rapidly after ischemia and trauma and to correlate temporally with the influx of neutrophils and the appearance of activated microglia and macrophages. Studies that use antibodies to block adhesion molecules have been shown to decrease neutrophil migration into CNS inflammatory sites after ischemia or spinal cord injury, which has been correlated with reduced lesion size. Such studies give support to the hypothesis that the robust influx of neutrophils into sites of CNS injury can contribute the tissue damage through the release of ROS and degradative enzymes that accompanies neutrophil activation. Such events also occur in the periphery, but these tissues are generally able to repair more readily than the CNS. These events can cause more permanent injury in the CNS, where axonal sprouting and regeneration are relatively inhibited compared with that of the peripheral nervous system.

The activation of microglia and the recruitment of macrophages into a damaged area of the CNS is delayed by a period of few hours to days compared with neutrophil influx. Significant presence of activated microglia/macrophages can be seen within 24 h, but the number of cells and amount of activation continues to increase for several days. Macrophages can be found in the tissue for several months after injury, particularly in areas of Wallerian degeneration that occur over a prolonged time course. As discussed previously, a great deal of controversy surrounds the issue of whether the presence of activated microglia and macrophages contributes to further damage or to tissue repair. The answer is likely a combination of both, depending on the type of injury and the amount of cellular activation that occurs.

Lymphocytes, particularly T cells, also can enter the CNS using adhesion-molecule interactions for extravasation across the endothelial cell barrier. In particular, interaction of the adhesion molecule VLA-4 on T cells with vascular cell adhesion molecule1 (VCAM-1) on endothelial cells is important for this process. T-cell entry into the CNS has been particularly associated with viral infections of the CNS and autoimmune processes. The most widely studied autoimmune disease of the CNS is multiple sclerosis (MS). The rodent model used extensively for this disease is experimental allergic encephalomyelitis (EAE), which is associated with CD4 $^{+}$ T-cell attack within the CNS. Studies have shown that activated or memory T-cells are particularly able to cross into the

parenchyma of the CNS. Their retention in the tissue is dependent on them interacting with their specific antigen. Without that interaction, the T cells appear to migrate out of the tissue again. Lymphocytic infiltrates found in this model are composed of T cells that are specific for various myelin peptide antigens and of antibody-producing B cells, along with some macrophages. The presence of lymphocytes is associated with areas of demyelination that result in loss of neural function. The initiating stimulus for MS and EAE is still not definitively known; however, there is support for a role played by an inflammatory response in the CNS (perhaps viral), allowing activated microglia to present myelin peptides to specific T cells. When paired with a peripheral inflammatory event that provides the cytokines and costimulatory signals needed for full T-cell activation, T-cell trafficking to the CNS and subsequent autoimmune damage to CNS myelin and oligodendrocytes can occur.

4.1. Role of Glial Activation in Enhancing Pain

Recent studies have shown an additional area of concern when spinal cord glia become activated: enhancement of pain associated with peripheral tissue inflammation, nerve damage, or damage to the spinal cord (Fig. 3). Under normal conditions (Fig. 3A), pain pathways from the periphery transmit a transient and adaptive signal of pain that is transmitted from the periphery on A-delta and C fibers that synapse in the dorsal horn of the spinal cord. Dorsal horn neurons, in turn, transmit the information to the CNS as discussed in detail in other chapters of this volume. At the level of the dorsal horn, the pain sensation can be modulated (enhanced or suppressed), but the pain subsides as the stimulus is removed or the trauma heals. One example of modulation that is adaptive is when hyperalgesia occurs as part of the “sickness response.” This appears to be mediated via a pathway that involves peripheral inflammatory cytokines signaling the brain as discussed previously in this chapter. This in turn

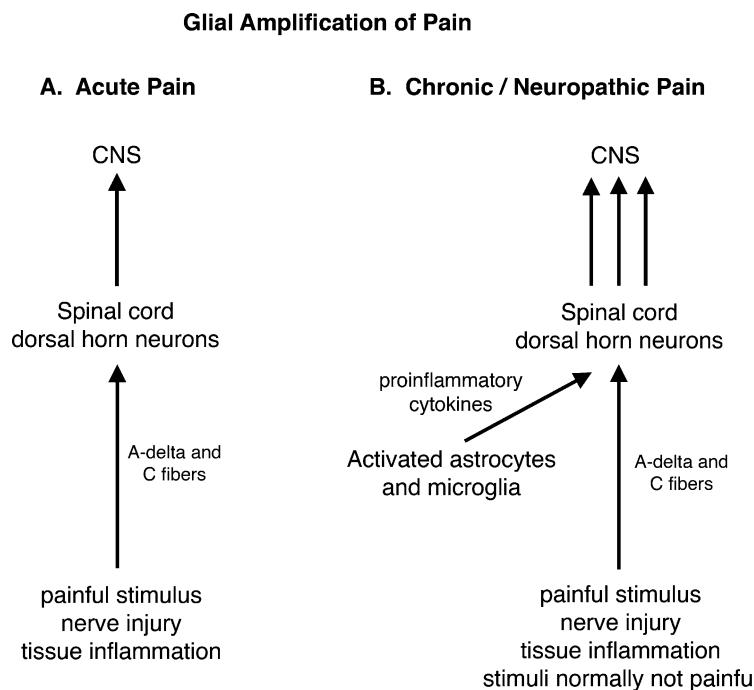


Fig. 3. Activated spinal cord glia contribute to maladaptive pain. **(A)** Under normal conditions of acute pain, information from painful stimuli are carried on A-delta and C fibers to the dorsal horn of the spinal cord. The appropriate dorsal horn neurons carry this information to the CNS for perception of pain. This pain pathway does not involve activated glial cells and terminates appropriately. **(B)** Chronic/neuropathic pain. Under certain conditions, glia (astrocytes and microglia) of the dorsal horn can become activated and release substances such as inflammatory cytokines that potentiate pain stimuli from the A-delta and C fibers. This results in a greatly amplified perception of pain that can be in response to stimuli such as light touch that are normally not painful or that can cause pain long after the initial injury has healed. Animal studies suggest that microglia are more important in the initial enhancement of pain, whereas astrocytes are involved with maintenance of the painful state. Therapies targeting activated glia could provide a new avenue for treating chronic pain.

stimulates production of cytokines such as IL-1 in the brain and subsequent activation of glia in the spinal cord resulting in enhancement of pain. In some conditions, however, pain becomes chronic and maladaptive, persisting beyond the initial condition that produced pain. In addition, some patients report pain that appears to come from areas of the body not initially involved, such as mirror-image pain from the opposite, unaffected part of the body. This type of pain is often resistant to effective treatment with drugs that affect the neurons of the pain pathways. Research has shown that spinal cord dorsal horn microglia and astrocytes become activated in response to tissue inflammation, peripheral nerve inflammation or damage, and after spinal cord injury. This glial activation may be related to neuron-to-glia communication. As discussed earlier, glial activation results in the release of inflammatory substances such as IL-1, TNF, and IL-6 (Fig. 3B). Numerous studies have shown that blocking the synthesis or activity of these cytokines can reduce or alleviate various models of allodynia and hyperalgesia. Thus, the evidence supports a role in the dorsal horn for glial-derived inflammatory substances in inducing neuronal hyperexcitability and exaggerated release of substance P and excitatory amino acids from presynaptic terminals. Furthermore, studies with different drugs to affect microglia versus astrocytes suggests that microglia have a more important role in the initial enhancement of pain, and astrocytes are key to maintenance of the painful state. Because glia do not appear to be involved in the normal acute pain pathway, drugs that target glia may provide effective means of treating chronic pain that does not respond well to classic neuropharmacologic therapies. The mechanisms and drugs being investigated in this promising area are discussed extensively in the suggested readings.

5. CONCLUSION

This chapter has shown that the CNS and the immune system are in constant communication. Under normal circumstances, this would help with the overall maintenance of homeostasis, as the nervous system and immune system work together in dealing with infections and damage. As shown schematically in Fig. 4, the major outflow systems from the CNS to the peripheral immune system are through direct neural and neuroendocrine systems. Hypothalamic connections to the spinal cord modulate outflow to the lymphoid tissues and sites of inflammation through the sympathetic nervous

system and neuropeptide systems. Modulation of lymphoid innervation has been shown to have major effects on inflammatory responses. In particular, catecholamine release from sympathetics has an inhibitory effect on innate immunity but can be inhibitory or enhancing to adaptive immunity. In addition,

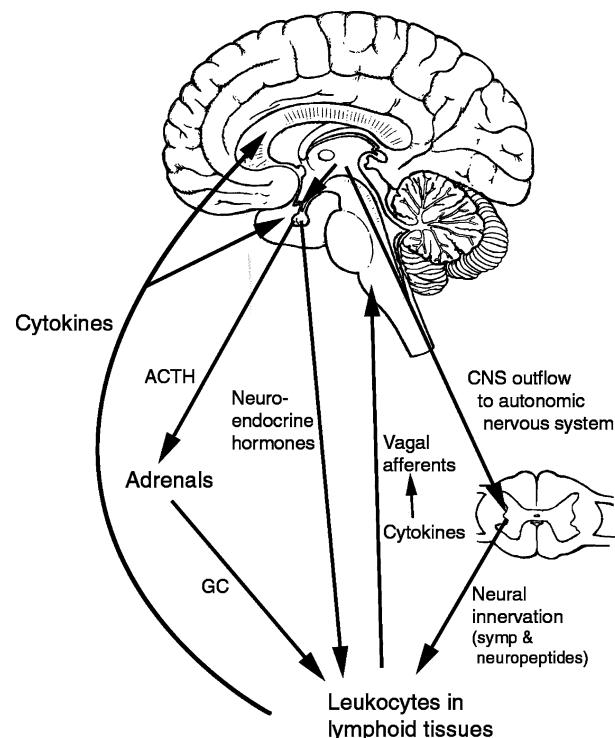


Fig. 4. Schematic of nervous system-immune system interactions. There are numerous routes of communication between the nervous and immune systems. Outflow from the CNS is predominately through the neuroendocrine system and through connections to sympathetic or neuropeptide innervation of lymphoid tissues. The hypothalamic connections to the pituitary can stimulate the release of various neuroendocrine hormones that can influence the immune system. In addition, inflammatory stimuli or stress can stimulate the release of adrenocorticotrophic hormone (ACTH), which in turn stimulates production of glucocorticoids (GC) from the adrenals, which can then influence the immune system in numerous ways. Hypothalamic output to the sympathetic preganglionics in the thoracic spinal cord or to other neuropeptide systems has also been shown to have a profound effect on immune function. Peripheral inflammatory responses can signal the CNS through blood-borne cytokines that are detected in CVOs or taken up through specific transporter systems. In addition, localized cytokines can stimulate afferents in the vagus nerve that can signal the brain stem and hypothalamus. Both routes can induce sickness behaviors that are mediated at the level of the CNS. The CNS itself can also produce cytokines that mediate inflammatory responses within the CNS or that affect efferent signals to the peripheral immune system.

hypothalamic stimulation of the pituitary and neuroendocrine system modulates the release of neuroendocrine hormones that can modulate immune function. In particular, stress, inflammation, or CNS cytokine-stimulated release of ACTH and glucocorticoids can strongly influence immune function. In turn, peripheral immune activation and release of cytokines can signal the CNS either directly or through vagal and musculocutaneous afferents. This in turn can stimulate prostaglandin and cytokine production in the CNS and initiate sickness behaviors in response to immune activation. Under normal circumstances, the coordinated response of the nervous and immune systems efficiently deal with infection and disease. Under certain conditions, however, inflammatory events in the CNS or alterations in the peripheral neural/neuroendocrine input to the immune system can become the basis of disease. It is clear that modified immune responses can occur in the CNS, particularly under conditions of disease or trauma, which can be necessary in recovery and clearance of debris. However, this immune response also can contribute to further damage. Activation of spinal cord glia has also been studied for their role in initiation and maintenance of maladaptive pain states. Given the complex-

ity of these two systems, much has yet to be learned of the normal role of neural-immune interactions and of the processes that could be effective targets in developing therapies for disease.

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Myasthenia Gravis

Gregory Cooper, Gerald Eichhorn and Robert Rodnitzky

Myasthenia gravis is the most common disorder of neuromuscular transmission. Patients experience weakness, with abnormally rapid fatigue of the muscles that move the extremities, face, eyelids, and eyes and the muscles required for swallowing and respiration. Involvement of these last two groups of muscles can be fatal if severe and untreated. Myasthenia is an autoimmune condition in which there is antibody-mediated alteration of the postsynaptic membrane of the neuromuscular junction and destruction of acetylcholine receptors. Although its clinical manifestations are protean, there are several useful serologic, pharmacologic, and electrophysiologic means of confirming the diagnosis.

ACETYLCHOLINE RECEPTORS IN MYASTHENIA GRAVIS

The Discovery of α -Bungarotoxin Began the Modern Understanding of the Acetylcholine Receptor

The discovery that a Taiwanese poisonous snake toxin, α -bungarotoxin, selectively binds to the nicotinic acetylcholine receptor at the neuromuscular junction provided a scientific tool that significantly enhanced efforts to understand the physio-anatomic basis of myasthenia gravis. Using radioactive α -bungarotoxin to label acetylcholine receptors, it was confirmed that their numbers are significantly reduced in myasthenic patients. It was demonstrated that inoculation of laboratory animals with acetylcholine receptor protein resulted in the development of an experimental form of myasthenia gravis, strongly suggesting an autoimmune basis for the illness. Antibodies against acetylcholine receptors can be demonstrated in most human patients with

myasthenia gravis, and their presence is a useful means of confirming the diagnosis.

Antibodies Can Affect Acetylcholine Receptor Functions in Several Ways

Several types of antibodies can be found in myasthenic patients. Approximately 90% of myasthenic patients with generalized weakness harbor one or more types of acetylcholine receptor antibody. In some patients, antibodies to the structural protein titin or the ryanodine receptor or to anti-muscle-specific receptor tyrosine kinase (MuSK) may be found. However, the most commonly found antibody binds to the acetylcholine receptor but does not block its receptive sites. The acetylcholine receptor has five constituent subunits arrayed around a central ion channel. The acetylcholine and α -bungarotoxin binding sites are separate, but they are both located on the two α -subunits of the receptor. Antibodies that do block the acetylcholine-receptive site are found in a much smaller percentage of patients and are often associated with more severe disease. Collectively, these abnormal antibodies accelerate the degradation of receptors by enhancing the normal process of endocytosis, and they impair the function of the remaining receptor by blocking their active sites and promoting a complement-mediated alteration of the architecture of the postjunctional membrane. The latter process includes widening of the synaptic cleft and smoothing of the normally infolded architecture of the postsynaptic membrane, with a resultant loss of receptor-containing surface area.

Acetylcholine receptor antibodies can be measured in serum. The specificity of circulating acetylcholine receptor antibodies for myasthenia gravis is high. However, false-positive results have been recorded

for a few persons without myasthenia gravis, such as those who received snake venom as a form of therapy, patients with tumors of the thymus, and those who received the drug penicillamine.

EPIDEMIOLOGY AND DIAGNOSIS OF MYASTHENIA GRAVIS

Myasthenia gravis occurs most often in young women and older men, peaking in the second and third decades in women and the sixth and seventh decades in men. Neonatal myasthenia gravis is a transient condition resulting from transplacental transfer of acetylcholine receptor antibodies from a myasthenic mother to her infant. As the antibodies are cleared by the affected infant, the myasthenic symptoms gradually resolve. This phenomenon confirms the importance of circulating antibodies in the pathogenesis of myasthenia gravis.

WEAKNESS AND EASY FATIGABILITY ARE THE CLINICAL HALLMARKS OF MYASTHENIA GRAVIS

Virtually any muscle in the body can become weakened in myasthenia gravis. Often, the first muscles to become involved are those responsible for movement of the eye or eyelid. Affected patients complain of diplopia and eyelid ptosis. Ocular symptoms are the presenting sign of myasthenia gravis in more than 50% of patients and ultimately affect more than 90% of patients. In a few patients, the symptoms remain confined to the eyes, resulting in the syndrome of ocular myasthenia gravis.

Appendicular, bulbar, and respiratory muscles are also commonly involved. In the arms and legs, proximal muscles tend to be involved more severely, resulting in difficulty arising from a chair, climbing stairs, or raising the arms over the head. Bulbar-muscle involvement results in symptoms such as difficulty in swallowing, nasal regurgitation of ingested fluids, and a characteristic nasal quality of the voice. Weakness of the facial muscles results in an inability to grimace, pucker the lips, or whistle. Involvement of respiratory muscles severely limits the volume of air that can be maximally moved in and out of the lungs.

One feature of myasthenic weakness that differentiates it from weakness caused by most other conditions is extreme and early muscle fatigability. Patients who ascend stairs may notice that the first few steps can be accomplished without difficulty, but that the next several steps become progressively more difficult.

SEROLOGIC, PHARMACOLOGIC, AND ELECTROPHYSIOLOGIC TESTING CAN HELP CONFIRM THE DIAGNOSIS OF MYASTHENIA GRAVIS

The usefulness of assaying acetylcholine receptor antibodies was previously discussed. Acetylcholine receptor antibodies can be demonstrated in only 90% of myasthenic patients with generalized weakness, and so other tests may be needed to confirm the diagnosis.

Pharmacologic testing involves the use of cholinesterase-inhibiting drugs. These agents improve myasthenic symptoms by preventing the normal hydrolysis and inactivation of elaborated acetylcholine in the neuromuscular synaptic cleft. The duration of the effect of elaborated acetylcholine is prolonged, allowing an increased number of interactions with the remaining acetylcholine receptors and, therefore, some improvement of neuromuscular transmission. Edrophonium (Tensilon) is a rapid and short-acting cholinesterase-inhibiting agent that results in a brief but striking improvement in myasthenic symptoms. After injecting it intravenously, marked improvement in symptoms can be demonstrated within minutes, which is helpful in confirming the diagnosis.

Electrophysiologic testing for myasthenia gravis consists of measuring the amplitude of a muscle's response to repetitive stimulation of its nerve. In normal persons, the amplitude of the muscle response remains constant during repetitive nerve stimulation at three cycles per second. In myasthenia gravis, the muscle response to stimulation at this frequency becomes progressively smaller, mirroring the fatigue experienced after repetitive limb movements. Typically, this decremental response can only be demonstrated in clinically weak muscles.

TREATMENT

With therapy, myasthenia gravis is seldom a fatal disorder. Cholinesterase inhibitors are useful in testing and in treating myasthenia gravis. Administered in proper amounts, they produce a mild improvement in muscle strength. If given in excessive amounts, they can produce side effects related to overstimulation of muscarinic acetylcholine receptors, including excessive salivation and tearing, diarrhea, and slowing of the heart rate. A similar cholinergic stimulation of muscle may paradoxically lead to increased weakness, presumably resulting from continuous end-plate depolarization. In its most extreme form, a

cholinergic crisis develops, in which there is marked weakness of the respiratory muscles.

Because cholinesterase inhibitors are only mildly effective, most severely myasthenic patients require immunotherapy. This approach, along with improved ventilatory support, has reduced the mortality rate for generalized myasthenia from the 40% to 70% reported before 1960 to less than 10% in 1994. One of the earliest immunologic therapies used for myasthenia gravis was removal of the thymus. In most myasthenia gravis patients, the thymus is microscopically hyperplastic, and in 20% of these patients, a tumor of the gland (e.g., thymoma) develops. Many myasthenic patients are improved after removal of the thymus, probably because of the gland's role in promoting and sustaining an autoimmune attack on acetylcholine receptors. The finding of acetylcholine receptor protein in the thymus supports this idea.

Immunosuppressive drugs, such as corticosteroids, azathioprine, cyclosporine, and mycophenolate mofetil, are extremely effective in inhibiting the aber-

rant immune response underlying myasthenia gravis. Often, as the patient improves on these therapies, there is a corresponding drop in the titer of antibodies to the acetylcholine receptor. A more direct way to lower this antibody titer is through the use of plasmapheresis. In this process, the patient's plasma, which contains the abnormal antibody, is removed and replaced with a plasma substitute. This procedure effectively lowers the titer of abnormal antibodies and can result in remarkable improvement until more antibody is produced. Intravenous immunoglobulin (IVIg) has also been used effectively.

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Degeneration, Regeneration, and Plasticity in the Nervous System

Paul J. Reier and Michael A. Lane

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1. INTRODUCTION

Neurotrauma and neurodegenerative disease often result in catastrophic disruption of the elaborate cellular interactions underlying the functional organization of the nervous system. As a result, devastating disabilities are endured that have been viewed for

many years as being irreversible. The first clinical report of traumatic brain and spinal cord injuries was found in an Egyptian papyrus (the Edwin Smith Papyrus) dating back more than 3500 years, which described both conditions as “an ailment not to be treated.” Recent basic science discoveries, however, have begun to temper centuries of pessimism. A challenge of current basic and clinical research is to define novel therapeutic approaches to promote functional repair and significant improvement in quality of life.

Contemporary medical neuroscience education thus warrants an appreciation for scientific advances in neurologic research and how preclinical progress may ultimately translate into future clinical applications.

In that context, the following discussion addresses basic principles underlying neural tissue reactions to trauma or disease and how these differ in peripheral (PNS) versus central nervous system (CNS) pathologies. Emphasis will then turn to spontaneous neural repair and intrinsic repair mechanisms (i.e., neuroplasticity), as well as an overview of potential therapeutic interventions designed to promote cellular rescue, regeneration, and neuronal replacement. For illustrative purposes, emphasis will be largely on neuronal changes occurring after blunt trauma (e.g., crush, contusion, compression) or severing of nerve fibers in the PNS or CNS. It should be recognized, however, that many cellular dynamics associated with neurotrauma can be exhibited in varying degrees under other neuropathologic conditions.

2. BASIC CELLULAR RESPONSES IN THE PNS AND CNS TO AXONAL DAMAGE: GENERAL PRINCIPLES

2.1. Axonal Injury Induces Anterograde Degeneration of Axons

The term *axotomy* refers to an interruption of axonal continuity. Thus, either transection or severe compression of a peripheral nerve or one or more CNS white matter tracts can result in a multitude of axons being divided into two cytoplasmic compartments. The portion of such interrupted axons that remains in continuity with the cell body is referred to as the *proximal segment* and is contained within the *proximal nerve stump*. The axonal region isolated from the neuronal soma is called the *distal segment* and is present within the *distal nerve stump* (Fig. 1).

Leakage of axoplasm from the proximal segment is an immediate aftermath of axotomy that can persist until healing of the cut end occurs by resealing of the axonal membrane. In addition, the cut end of the proximal segment can undergo degenerative retraction from the original site of injury—a phenomenon referred to as *proximal (segment) die-back* (Fig. 1). Such proximal axonal breakdown is usually limited in distance (up to the preceding node of Ranvier), unless the lesion occurs close to the cell body, in which case proximal die-back can advance to the soma and thus result in *retrograde neuronal degeneration* (discussed in more detail later in this chapter).

Otherwise, the main consequence of axotomy is a progressive demise of the distal axonal segment. This phenomenon, referred to as *Wallerian* or *anterograde degeneration* (Fig. 1 and Fig. 2), reflects the fact that the metabolic maintenance and integrity of the axon is primarily dependent on synthetic activity in the cell body and anterograde axonal transport (see Chapter 1). Thus, once separated from the cell body, the distal axonal segment does not have sufficient inherent biosynthetic capacity to maintain itself.

Cytologic features of Wallerian degeneration often appear within a day after injury and include significant axonal swelling, abnormal accumulations of axonal organelles followed by dissolution of cytoskeletal components (e.g., microtubules and neurofilaments; see Chapter 1), and ultimate axonal collapse often leaving hollowed-out myelin profiles (Fig. 2). A considerable degree of axonal breakdown is caused by an unregulated influx of calcium, which leads to the activation of calcium-dependent proteolytic enzymes that cause degradation of the cytoskeleton. Loss of cytoskeleton and concurrent disruption of the integrity of the axolemma contribute most directly to axonal fragmentation and collapse.

Wallerian degeneration also entails a secondary loss of the myelin sheaths surrounding degenerating axons (Fig. 1 and Fig. 2). The association of such myelin pathology with axonal disintegration implies an intimate dependency of myelin-producing Schwann cells (in the PNS) and oligodendrocytes (in the CNS) on axonal integrity. This principle is a corollary of development, during which the axon dictates whether myelin is formed and the ultimate thickness of the sheath to be established.

A concomitant loss of axonal terminals (e.g., *terminal degeneration*) is also associated with anterograde degeneration. Functionally, axonal damage results in a rapid loss of synaptic transmission, which can occur before the first visible cytologic signs of terminal disruption. For example, damage to axons from spinal motoneurons results in breakdown of the neuromuscular junction and phagocytosis of debris by Schwann cells over the course of a few days post-axotomy. Transmission at the neuromuscular junction ceases, however, within a few hours of nerve damage.

Injury to a given peripheral nerve can thus lead to the anterograde degeneration of large populations of axons and their surrounding myelin sheaths, which systematically progresses from the lesion to presynaptic endings. It is important to appreciate, however, that Wallerian degeneration *only* applies to the axon and its contents. The distal nerve stump

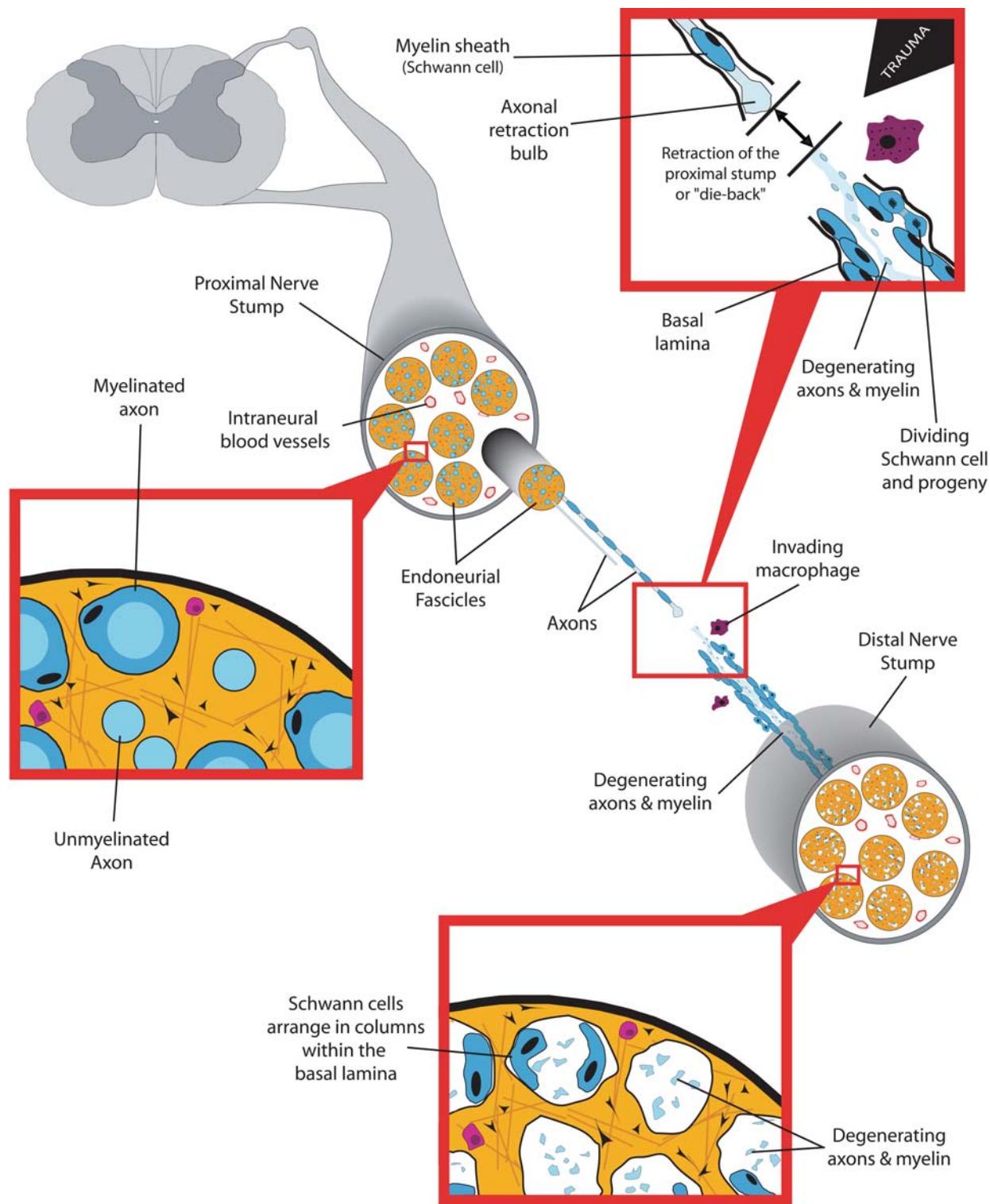


Fig. 1. A schematic diagram showing the sequence of events that leads to Wallerian (anterograde) degeneration. The normal cytology of a peripheral nerve is shown as a point of reference (expanded inset A). After axonal injury, the proximal stumps retract from the site of injury forming distinctive “retraction bulbs” (expanded inset B). Meanwhile, the distal portion of injured axons degenerate, but all other elements of the peripheral nerve remain intact (expanded inset C). Thereafter, Schwann cells begin to proliferate, and blood-borne macrophages infiltrate the degenerating nerve stump and assist Schwann cells with phagocytosis of axonal and myelin debris (expanded inset B). Schwann cells then become arranged in columns known as the “bands of Büngner” within common basal laminae (expanded inset C). Such Schwann cell units provide a cellular pathway along which regenerating axons extend distal to the site of injury (see also Fig. 10) (see Color Plate 2, following p. 378).

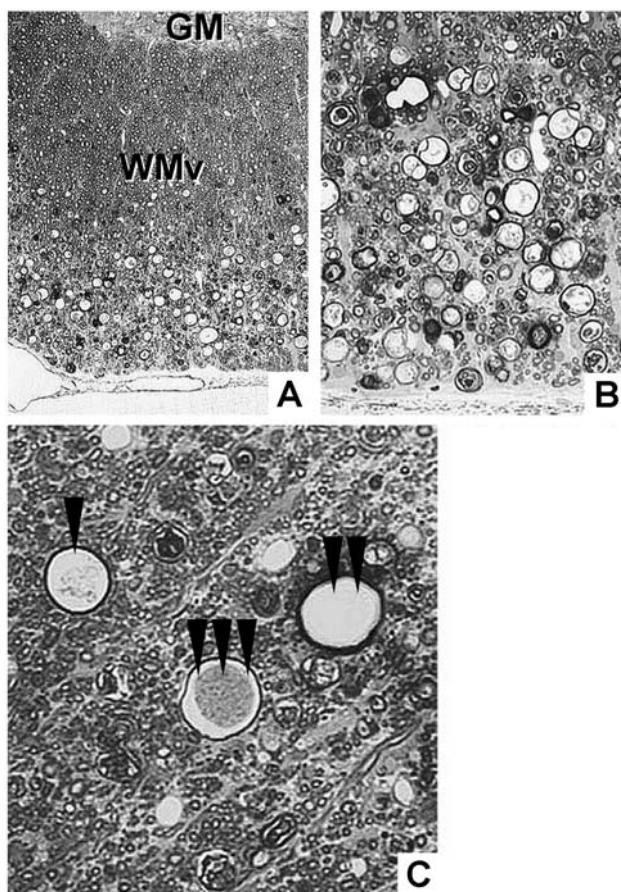


Fig. 2. Histologic views of Wallerian degeneration caudal to an experimental spinal cord injury. **(A)** A partial cross-sectional view of a rat spinal cord several segments below the lesion site. The outer rim of ventral white matter (WMv) shows a distinct degenerative “foamy” appearance in contrast with those normal-appearing myelinated fibers more immediately surrounding central gray matter (GM). Because neurons giving rise to these myelinated axons are located at suprasegmental levels above the injury level, the axonal degeneration shown is “distal” to the injury. **(B, C)** Higher-magnification views of degenerating white matter showing myelin breakdown and axonal swelling. Various stages of axonal degeneration are further illustrated in **(C)**. Initially, many axons enlarge (single arrow) and show accumulations of organelles, such as mitochondria, which appear as dot-like structures in the axoplasm. At the ultrastructural level, numerous lysosomes and dense accumulations of cytoskeleton would also be seen. The axon continues to break down (double arrows) leaving behind a hollowed out myelinated profile (triple arrows). Subsequently, such structures collapse, and myelin debris is removed by macrophages.

itself does not degenerate. Vascular and connective tissue components of the nerve remain along with cohorts of Schwann cells (Fig. 1), the significance of which will be discussed shortly.

2.2. Axotomy Induces Responses at the Level of the Neuronal Cell Body

In addition to axonal pathology, effects of axotomy can be exhibited at the level of the cell soma in the form of metabolic, molecular, cytologic, as well as neurophysiologic changes. The cell body response depends on many variables, including:

- the age of the subject at the time of injury
- the distance of the lesion from the cell body
- differences between animal species
- the nature and severity of the lesion
- the extent of axonal collateralization between the site of axotomy and the cell body
- the functional type of cells involved
- the PNS or CNS location of the axotomized neuronal populations

For these reasons, it cannot be generalized in what way any given neuronal population will respond to axotomy. Although many possibilities have been proposed for how axonal damage triggers metabolic changes in the cell soma, it is likely that multiple, temporally related signals are involved. One of the more immediate and plausible signals of injury is a massive neuronal depolarization due to influxes of calcium and sodium ions at the site of axotomy. Such pronounced electrical feedback activates a large variety of molecular responses at nuclear and cytoplasmic levels of the neuron.

Axotomy also leads to an interruption in the retrograde axonal transport of target-derived molecules to the cell body. The absence of such molecular feedback can likewise prompt neuronal biosynthetic changes. This principle was demonstrated by experiments using intact axons in which only retrograde transport was blocked. By preventing the flow of target-derived molecules, this procedure resulted in upstream cell body responses similar to those that would otherwise appear after axonal injury.

A classic cytologic example of a cell body response to axotomy is *chromatolysis*. Its characteristic features include formation of cytoplasmic vacuoles, nucleolar enlargement, displacement of the nucleus from a central to an eccentric or peripheral cell body location beneath the cell membrane, and swelling of the cell body (Fig. 3). A prominent feature of chromatolysis observed under the light microscope is the dissolution of Nissl substance—the ultrastructural correlate of which is fragmentation of rough endoplasmic reticulum with a concomitant increase in the density of free polyribosomes, the appearance of which is consistent with elevated RNA metabolism.

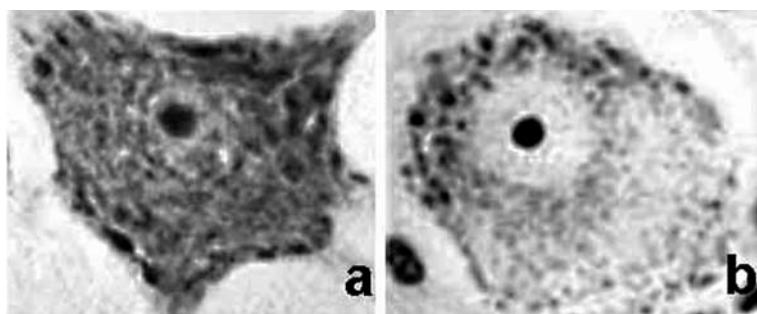


Fig. 3. Images of cresyl violet stained neurons. **(a)** A normal neuron exhibiting Nissl bodies (i.e., rough endoplasmic reticulum at the ultrastructural level) throughout its cytoplasm. **(b)** An example of chromatolysis showing the transition from a normal neuronal soma with clusters of Nissl substance to a swollen cell body with a displaced and more lightly stained nucleus and a reduced density of Nissl bodies.

and protein synthesis more typically seen in young neurons. It should be noted that chromatolysis is not a universal neuronal response, but one that is exhibited only by certain neuronal populations and subject to many variables. Whereas certain features of chromatolysis are consistent with features signaling imminent cell death (e.g., nuclear changes, cytoplasmic vacuolation, and cellular swelling), the appearance of polyribosomal clusters, seen in normally developing neurons, is more consistent with anabolic responses supporting regeneration.

2.3. The Fate of Axotomized Neurons Can Follow One of Three Basic Paths

After axonal injury, some neurons that will survive can shift their metabolism toward the biosynthesis of molecules associated more with cellular survival and repair rather than constituents more typically associated with normal neuronal function. An example is neurotransmitter synthesis, which is strikingly decreased after nerve injury, whereas synthesis of certain cytoskeletal and growth-related proteins is upregulated. Alternatively, there are instances in which neurons survive but show a significant reduction in cell body size (i.e., *atrophy*) and limited, if any, anabolic responses to axotomy (Fig. 4A). This state of “cellular limbo” is often believed to be associated with overall neuronal dysfunction although more definitive evidence remains to be obtained.

A third consequence of axonal damage is that some neurons may undergo regressive changes that could cascade into molecular events directed at neuronal death (e.g., *programmed cell death* or *apoptosis*) as will be discussed in more detail later. Thus, some neurons that are unable to survive axonal damage succumb to what is referred to as *retrograde cell death* (Fig. 4B). For example, after experimental

lesions that interrupt ascending dorsal spinocerebellar axons, neurons from which those axons arise in the dorsal nucleus of Clarke undergo progressive retrograde degeneration (Fig. 5). There are many factors to which loss of neuronal viability after axotomy can be attributed (see later), and as a general rule lesions occurring close to the cell body of origin usually result in a greater chance of cell death.

Cell death also can occur in neuronal populations that are synaptically associated with damaged neurons. For instance axotomy, with or without ensuing cell death, can lead to a phenomenon referred to as *anterograde* (orthograde) *transneuronal* or *trans-synaptic degeneration*. As a result of Wallerian degeneration, postsynaptic neurons become at least partially denervated. Under certain circumstances, such a reduction in presynaptic input can then precipitate an eventual degeneration of the target cell (Fig. 4C). A frequently cited example of anterograde transneuronal degeneration is in the lateral geniculate nucleus (LGN) of the thalamus, in which the postsynaptic cells are significantly deafferented after damage to the optic nerves. An analogous situation can be seen with regard to peripheral target structures, such as muscle, where denervation atrophy usually follows a peripheral nerve injury (Fig. 6).

Conversely, neurons that synapse with axotomized cells can die by virtue of *retrograde transneuronal* or trans-synaptic degeneration (Fig. 4D). An important corollary of these transneuronal degenerative responses to axotomy is that similar principles apply to many neurodegenerative diseases. For instance, the loss of certain neuronal populations that are the primary targets of a particular neurologic disorder could lead to more widespread cell degeneration elsewhere in the nervous system as a result of deafferentation (e.g., orthograde transneuronal degeneration)

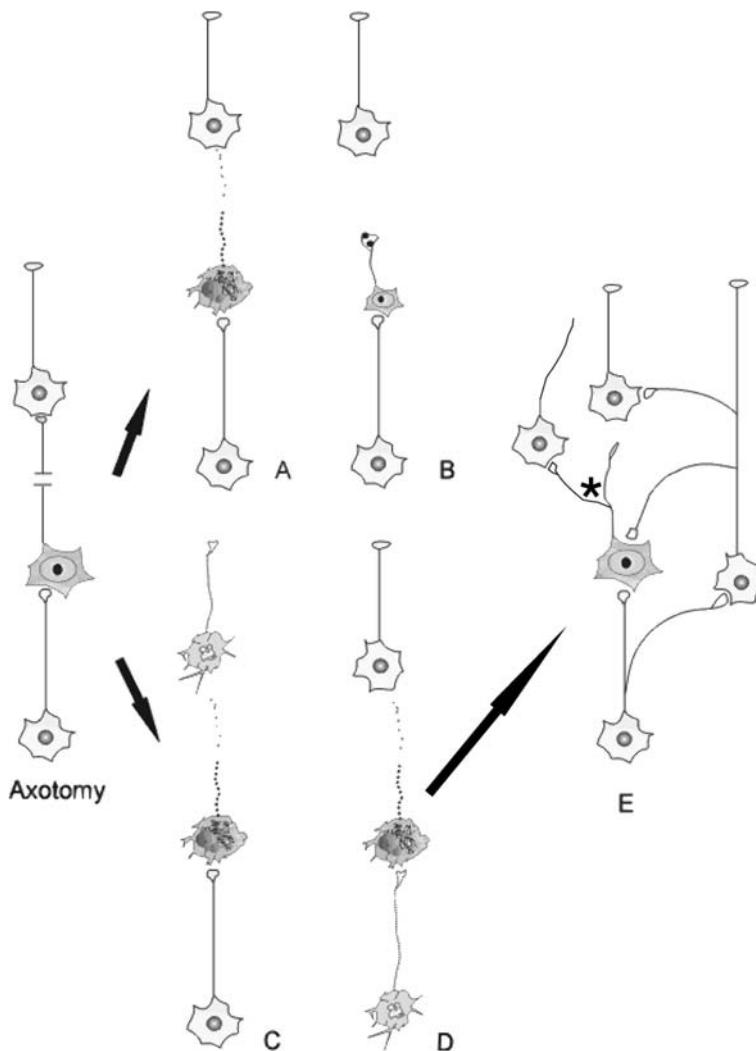


Fig. 4. Several basic neuronal responses to injury are illustrated in this diagram (see text). Three neurons are shown on the left, with the middle cell depicted as having been axotomized. **(A)** The progressive demise of a neuron after axonal interruption is referred to as retrograde cell death. **(B)** In other nonregenerative circumstances, the injured neuron survives, but undergoes a retrograde atrophy. **(C)** Wallerian degeneration and resulting deafferentation of postsynaptic neurons can result in anterograde transsynaptic or transneuronal cell death. **(D)** Conversely, death of the axotomized neuron can lead to a retrograde transneuronal degeneration of neurons originally presynaptic to the injured neuron. **(E)** Rescue of neurons that can be directly or indirectly affected by axotomy may occur in a variety of ways. One that is illustrated is by virtue of trophically sustaining inputs from uninjured cells that project onto the vulnerable neurons or by way of collateral projections from cells that are at risk of neuronal death or atrophy (asterisk).

or the loss postsynaptic of targets (e.g., retrograde transneuronal degeneration). One illustration of this point is Huntington's disease in which pathology is not solely restricted to the striatum but also extends in terms of neuronal loss into other regions of the basal ganglia, as well as into cortical regions during progression of the disease. Such more global transneuronal cell death also explains the spectrum of associated behavioral and cognitive consequences of many neurodegenerative diseases.

2.4. Neuronal Fates Can Be Defined by Changes in Nutritive Support

These examples of primary and anterograde/retrograde trans-synaptic (a.k.a. *transneuronal*) cell degeneration underscore the important concept of *neurotrophism*. This principle refers to the establishment of sustaining molecular interactions between either neurons or neurons and their peripheral target structures. For instance, denervation neuromuscular atrophy (Fig. 6) can be caused by a loss of muscle activity

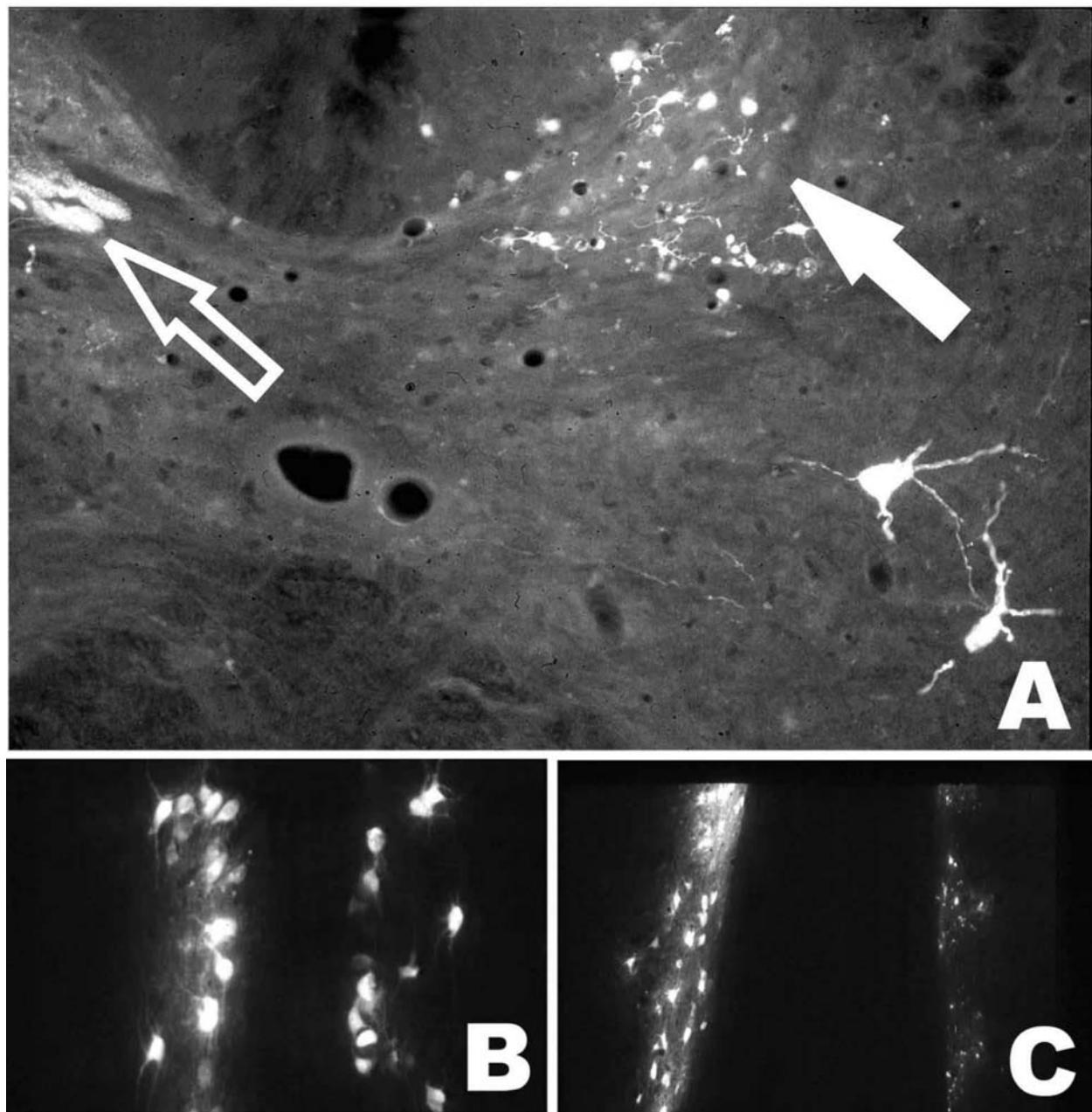


Fig. 5. Shown is an example of *retrograde cell death* in the dorsal nucleus of Clarke (e.g., neurons that give rise to dorsal spinocerebellar axons) of the adult rat spinal cord. This histologic cross-section was obtained at the L2 spinal level several weeks after a right-sided hemisection of the spinal cord was made at T11. Prior to that procedure, neurons projecting to the cerebellum were prelabeled by retrograde transport of a fluorescent dye that was injected into appropriate regions of the cerebellum. The tissue sections were then viewed and photographed by fluorescence microscopy. (A) A small cluster of spinocerebellar neurons (*open arrow*) is seen on the uninjured side of the spinal cord, whereas only fluorescent debris in small cells with ramified processes (*solid arrow*) can be seen in the same region on the injured *right side*. (B) A horizontal section of a normal spinal cord showing bilateral labeling of dorsal spinocerebellar neurons. (C) A comparable section from an animal with a right-sided hemisection that severed dorsal spinal cerebellar axons. Note the absence of large neurons on the *left* and the presence of microglial elements that had ingested the label (*see also Fig. 19*).

in part, as well as an absence of neuronal “nutritive” or trophic support to denervated muscle(s). In similar ways, diminished synaptic drive or neurotrophic

support, which are not mutually exclusive, can account for anterograde transneuronal degeneration or neuronal atrophy (Fig. 4C). Alternatively,



Fig. 6. Prominent muscular atrophy is seen in this individual's forearm with suboptimal regeneration after peripheral-nerve injury at the level of the brachial plexus. Although the ability to flex the arm has recovered, the overall degree of functional recovery is minimal. This illustrates an example of the absence of trophic interaction between nerve and muscle. (Illustration kindly provided by Dr. Susan E. Mackinnon, Washington University School of Medicine and Barnes-Jewish Hospital.)

the death of an axotomized cell can contribute to neurotrophic imbalances that affect neurons that synapsed with it, thereby leading to retrograde transneuronal degeneration or atrophy (Fig. 4D).

Trophic substances that are derived from the target cell are normally conveyed to the cell body of the presynaptic element by retrograde axonal transport. This principle also applies to retrograde degeneration or atrophy of axotomized neurons, which are no longer in contact with their targets (e.g., sources of trophic support) because of axonal interruption and failure to reestablish such connections. However, retrograde degeneration/atrophy may be curtailed under some conditions by trophically effective inputs from other cells onto the injured neuron. Sustaining afferent inputs also may prevent anterograde and retrograde transneuronal degeneration (Fig. 4E). The axotomized cell also may have collaterals emerging from the proximal axon segment, thus retrogradely obtaining other

Table 1
Molecules Shown to Mediate Axonal Growth

	<i>Growth-promoting factors</i>		<i>Growth-inhibiting factors</i>
Neurotrophic factors	Work done in the mid-1900s using cell cultures discovered a molecule produced by the mouse sarcoma that promoted nerve growth (nerve growth factor; NGF). Since this discovery, molecules produced by a range of cells have been discovered that promote growth in the nervous system (e.g., FGF-2, NT-3, NT-4, BDNF, CNTF, GDNF).	Chondroitin sulfate proteoglycans (CSPGs)	Several CSPGs have been identified (e.g., neurocan, NG2, phosphacan, brevican, and versican), each inhibitory to axonal growth.
Extracellular matrix (ECM) molecules	ECM molecules have been shown to have growth-promoting, migration, and even differentiation-promoting properties. Two key examples of these are laminin and fibronectin.	Tenascin	Tenascin subtypes C, R, and X have all been shown to inhibit axonal growth in the CNS.
Guidance molecules	Guidance molecules act on the axonal growth cone to direct growth toward an appropriate target (attraction) or away from an inappropriate one (repulsion). Several guidance molecules have been identified over the past 20 to 30 years, including netrin, slit, semaphorin, and ephrin. There are also several subtypes of these molecules.	Myelin and the product of myelin degradation	Myelin in the CNS and products that arise from its breakdown have been shown to inhibit axonal growth. Three main proteins have been identified: myelin-associated glycoprotein (MAG), oligodendrocyte-myelin glycoprotein (OMgp), Nogo.
	Guidance molecules		Depending on the subtype and the receptor that it acts on, guidance molecules may also have repulsive properties. For instance, the ephrin A subtype is repulsive, whereas ephrin B is attractive.

postsynaptic sources of trophic support. Depending on the extent of collateralization and amount of additional target-derived trophic resources available, these collaterals could enable the survival of a damaged cell, although it may still exhibit some degree of atrophy.

Neurotrophic factors are molecules that play important roles relative to cell differentiation, survival, and growth in the developing nervous system. This is exemplified by nerve growth factor (NGF)—a member of a neurotrophin family of molecules that also includes brain-derived neurotrophic factor (BDNF), and neurotrophins NT-3 and NT-4/5 (Table 1). Broadly stated, neurotrophic factors also are important regulators of neuronal function in the adult nervous system and can have considerable impact on neuronal responses to injury.

For example, NGF and other neurotrophic factors have been shown to have profound maintenance and axonal growth-promoting effects on certain neuronal

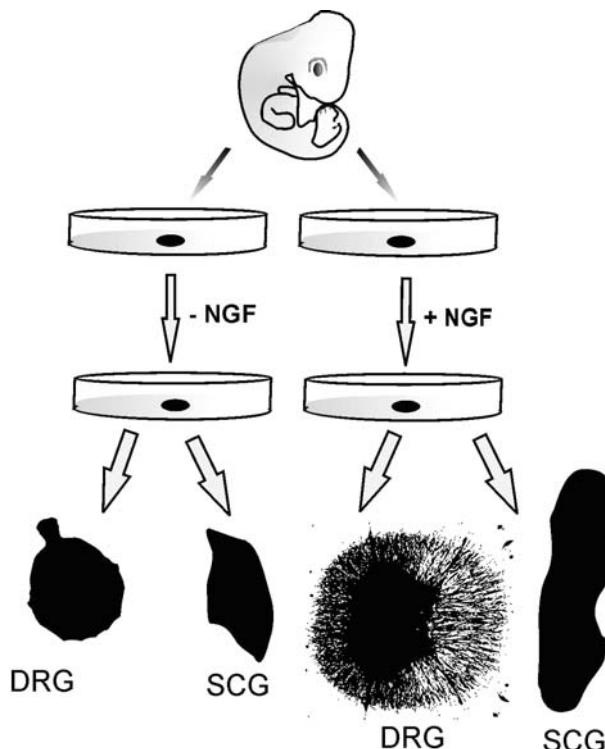


Fig. 7. Two experimental examples are illustrated of neurotrophic action using NGF as the model neurotrophic factor. Dorsal root (DRG) and superior cervical ganglia (SCG) are dissected from the embryo (such as the rat, mouse, or chicken) and then grown in tissue culture in either the presence or absence of NGF. In the case of the DRG example, NGF stimulates extensive fiber outgrowth as seen on the right, thereby demonstrating the neurite-promoting action of this factor. The SCG diagrams provide an example of NGF's effect on neuronal survival and growth, as exhibited by the larger size of the treated SCG explant.

populations in the normal or injured adult CNS, as well as on neuronal maturation during development (Fig. 7). As one illustration of this principle in the mature CNS, cholinergic neurons in the septal region of the basal forebrain are highly dependent upon NGF for their overall survival. Transection of the fornix results in the interruption of the axonal projections from these cells to the hippocampus where cells represent a source of NGF. Consequently, many axotomized cholinergic neurons in the septum either die or exhibit severe atrophy and loss of their cholinergic phenotype (Fig. 8A, B). The fate of these vulnerable

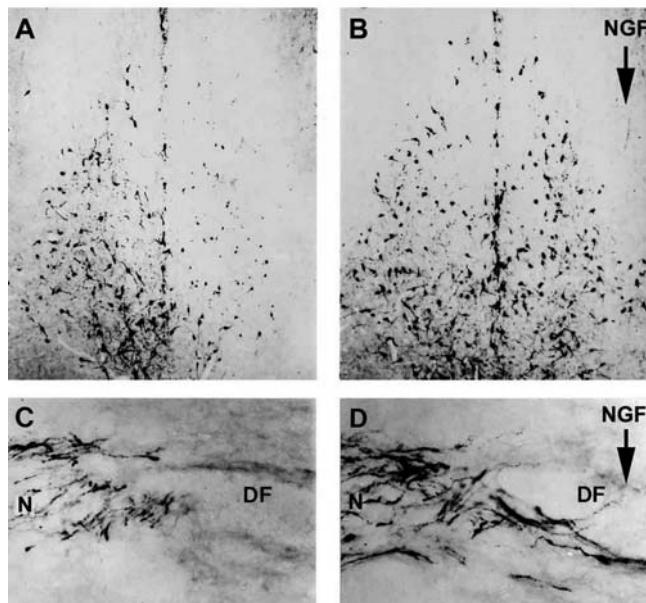
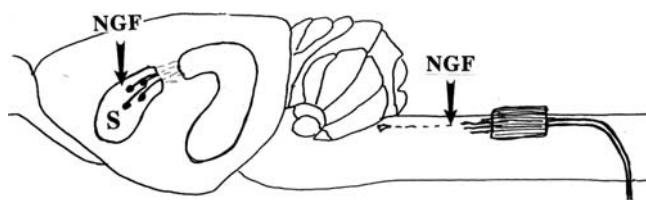


Fig. 8. Nerve growth factor (NGF) has been used to prevent degeneration of basal forebrain cholinergic neurons including those of the septum (S) after traumatic lesions and to promote regeneration of sensory axons after spinal cord injury, most often in rodent models. (A) Chronic treatment with control solutions does not prevent the disappearance of ChAT-positive (cholinergic) neurons after a lesion on one side of the brain, but (B) NGF does. (C) Sensory axons that have grown vigorously across a peripheral nerve bridge (N) do not reenter the dorsal funiculus (DF) white matter of the spinal cord, as it is nonsupportive. (D) After NGF infusion, many sensory fibers can extend into that white matter tract over millimeters, responding to the chemoattractant properties of NGF. (Figure kindly provided by Dr. Theo Hagg, University of Louisville.)

cells, however, can be reversed by infusion of exogenous NGF. Because cholinergic septal neurons appear to degenerate in Alzheimer's disease, experimental findings of this nature have suggested provocative therapeutic possibilities involving NGF delivery to rescue these neurons. Similarly, another neurotrophin, glial cell-derived neurotrophic factor (GDNF), can rescue dopamine-producing neurons in the substantia nigra after experimental lesions, and these findings have led to some clinical trials directed at the treatment of Parkinson's disease.

3. NEURONAL RESPONSES TO AXONAL INJURY IN THE PERIPHERAL NERVOUS SYSTEM

3.1. Neurons Associated with the PNS Can Exhibit Robust Regrowth of Damaged Axons

Cell death after peripheral nerve injury is dependent upon several variables, the most prominent of which are the neuronal cell type(s) affected, proximity of the lesion to the cell body, type of injury, and age-related factors. For example, large dorsal root ganglion cells and some peripheral and spinal preganglionic neurons of the autonomic nervous system are more susceptible to peripheral nerve injuries than are motoneurons. However, motoneurons become more vulnerable after spinal root avulsions, which can occur with cervical spinal cord injuries due to large tensile forces on nerve roots. Such injuries not only cause interruption of motor axons but also damage to motoneuron collaterals within the spinal cord. Otherwise, neurons located exclusively within the PNS or those that send axons from the CNS into peripheral nerves (e.g., spinal or cranial motoneurons) are fairly resilient and have the innate capacity to support axonal regrowth, even for long distances. In the case of dorsal root ganglion cells, this principle applies to peripherally more than to centrally directed processes for reasons to be discussed later. The onset of axonal regrowth does not occur immediately after axotomy, and the length of delay depends on variables such as the severity of the injury or the proximity of the lesion to the cell body. An important consideration is that robust axonal regeneration is not necessarily synonymous with functional recovery. Instead, the degree and quality of functional return is highly dependent on the ability of axons to reconnect with their proper targets (i.e., reinnervation, or sometimes referred to as functional regeneration).

3.2. Regeneration Is Initiated by the Formation of Growth Cones That Possess Selective Preferences for Certain Tissue Constituents

At the preserved end of the proximal nerve segment, morphologic changes occur that initially lead to the formation of multiple axonal sprouts from each damaged axon. Appearing at the end of each sprout is an amoeboid-like structure referred to as the growth cone (Fig. 9A, B). Growth cones are

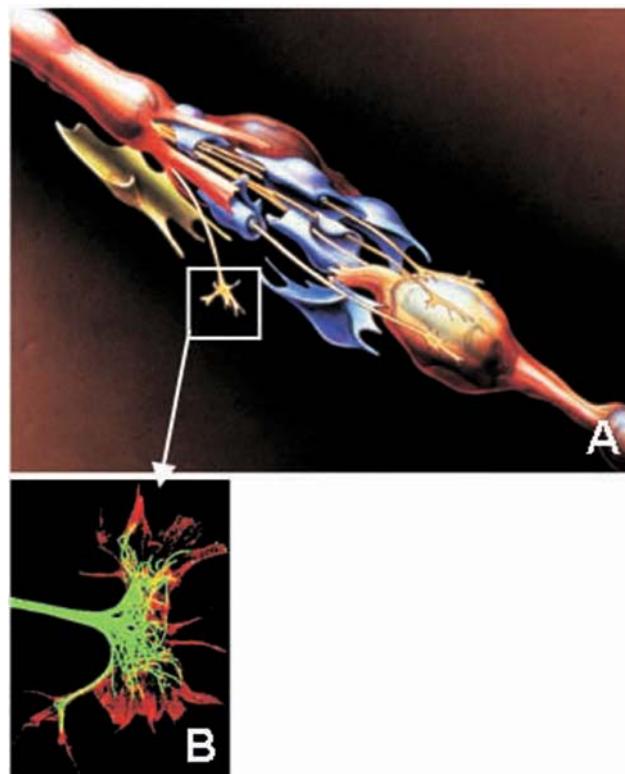


Fig. 9. (A) A three-dimensional rendition of early axonal regeneration after peripheral-nerve damage. Growth cones (e.g., *boxed profile*) are seen extending into the lesion gap (*blue profiles* representing connective tissue elements), and some make contact with Schwann cells (*red profiles*) in the distal stump. (Drawing kindly provided by Dr. Susan E. Mackinnon, Washington University School of Medicine and Barnes-Jewish Hospital.) (B) An axonal growth cone of an embryonic chick sensory neuron is shown. The growth cone is doubly stained with an antibody against tubulin (*green*), which labels microtubules, and rhodamine-phalloidin to label actin filaments *red*. The bundle of axonal microtubules (*green*) splays apart in the growth cone, and individual microtubules extend forward to interact with actin filament bundles and networks. These interactions between actin filaments and microtubules are important in determining directions of axonal growth and branching (see text). A small axonal sprout has formed at the lower left margin of the growth cone. (Figure generously provided by Paul C. Letourneau, Ph.D., University of Minnesota.) (see Color Plate 3, following p. 378).

highly specialized motile structures that when observed in tissue culture move in a crawling fashion by extending and retracting cytoplasmic extensions (e.g., filopodia) or flattened veil-like projections (e.g., lamellipodia). The proximal nerve sprouts increase in length as the growth cones advance, and their formation represents the earliest overt phase of axonal regeneration. While growth cone structural integrity and mobility appear to be highly dependent upon axonal transport of constituents synthesized in the cell body, recent evidence indicates that significant local axonal synthesis also may be involved.

In addition to their mobility, growth cones have the remarkable capacity to sense and respond to cues in the surrounding tissue milieu. They have affinities for some molecules and are repulsed, arrested, or collapsed by others. As growth cones advance, they extend their delicate filopodia, which have receptors for various tissue-associated ligands. Using these receptors, filopodia attach to favorable or growth-promoting and permissive substrates. This leads to a mechanism within the growth cone itself for allowing it to continue advancing. Alternatively, if filopodia encounter adverse tissue environments, their initial attachments are unstable, they retract, and the growth cone is arrested in its advance (i.e., growth cone collapse) or turns in the direction of those filopodia that have found more favorable substrates.

The mechanisms of growth cone movement are complex and in several respects parallel those that underlie motility in other cell and tissue types. Briefly stated, movement of growth cones has been associated with certain cytoskeletal constituents—most notably actin and myosin II. Actin is intimately involved in the extension of filopodia (Fig. 9B), and myosin II appears to underlie the traction-based movement of growth cones. Guidance cues seem to influence growth cone trajectories by modulating the rate and location of actin polymerization via intracellular signaling mechanisms that appear to be mediated by transient elevations of intracellular calcium.

As noted above, growth cones have selective chemoaffinities for molecules that attract and induce them to form stable attachments. Proteins that lure growth cones are *neurotropic*. Although this term has a meaning different from *neurotrophic*, it is important to note that in some cases a neurotrophic factor also can exhibit neurotropic properties. Tissue culture experiments have shown, for example, that the direction of growing neurites can be altered by minute deposits of NGF placed at varying distances from their growth

cones. Other molecules that have neurite-growth-promoting properties include a variety of extracellular matrix (ECM) molecules—most notably laminin and fibronectin—and various cell adhesion molecules (Table 1). Many of these substances bind with specialized receptors on the growth cone (e.g., integrins), which can then result in calcium-ion influx, thereby affecting the stability and directionality of growth cone attachments.

The important point to consider in this abbreviated overview of growth cone biology is that these structures are very dynamic. They have the ability to sample various cellular environments and possess inherent sensitivities to different molecules that are critical to the overall success of regeneration. Thus, in addition to the injured neuron's intrinsic ability to initiate a growth response after axotomy, axonal regeneration also is highly dependent on how the growing tip of the regenerating nerve fiber interacts with different molecular, physiologic, and physical cues as it navigates toward target sites.

3.3. The Nature of the Cellular Environment at the Site of Nerve Damage Can Influence the Ultimate Success of Regeneration by Affecting Growth Cone Behavior

As growth cones emerge from the proximal nerve stumps, they first encounter the cellular and molecular microenvironment of the lesion site, which can vary considerably depending on whether nerve damage was caused by severe compression or transection. The setting after a nerve crush stands in stark contrast with what growth cones must encounter after nerve transection, which gives rise to a gap between the retracted proximal and distal cut ends. An extended lesion site usually presents a very unfavorable environment for directed fiber outgrowth. Proliferating cells from the proximal and distal stump endoneurium and epineurium, as well as other surrounding non-neural tissues, infiltrate the wound site and form a tortuous terrain. In some cases, scarring can be so dense that the nerve ends become blindly embedded in a densely collagenous fibrotic tissue. In other cases, axons can wander and may never make contact with the distal nerve stump. The chances of regenerating fibers reaching the distal nerve stump become progressively less as a function of increasing lesion gap distance, thereby negating the potential for any useful regrowth beyond the injury. Human data indicate that wound gaps >4 cm lead to negligible distal regeneration and functional recovery.

Scarring at the wound site can likewise promote development of highly disorganized axonal terminal enlargements at the cut edge of the proximal nerve stump. Such swollen structures often contribute to a tangled meshwork of profusely branched regenerated nerve fibers, Schwann cells, fibroblasts, and collagenous matrices. Collectively, this tissue matrix constitutes a posttraumatic *neuroma*, which can severely impede further regeneration and, even more importantly, be a source of painful stimuli. The latter is due to the close approximation of fine-caliber fibers, which enables the spread of electrical current from one fiber to another. This is referred to as *ephaptic transmission*, which can evoke disordered sensory phenomena.

3.4. Cellular Responses and Regenerative Dynamics Distal to a Nerve Injury

3.4.1. SCHWANN CELLS PROVIDE A PERMISSIVE SUBSTRATE ESSENTIAL FOR AXONAL ELONGATION AND ARE RESPONSIBLE FOR MYELINATION OF REGENERATED AXONS

Within the first 24 h after injury, Schwann cells in the distal nerve stump begin proliferating and reach a peak of mitotic activity by approximately 3 days after injury with cell divisions continuing for 2 to 3 weeks with a time-dependent decline in frequency. Dividing Schwann cells and their progeny become organized into characteristic linear arrays (called the *bands of Bunger*) that are aligned virtually parallel to the initial trajectory of the axons they had surrounded (Fig. 1 and Fig. 10). In addition to what occurs in the distal nerve stump, Schwann cells at the separated ends of the distal and proximal stumps often infiltrate the lesion gap. This can result in a restoration of Schwann cell continuity between the proximal and distal cut ends. How extensively Schwann cells migrating from the two separated ends meet depends on the length of the gap and degree of scarring. In contrast, the distribution of Schwann cells at a crush site is virtually identical to that seen more distal to the lesion for reasons noted below.

Unless otherwise hindered by adverse conditions in the lesion, by as little as 3 to 5 days after injury regenerating fibers reach the distal stump where they encounter the terrain of linearly oriented Schwann cells, which have long been considered to have a significant role in PNS regeneration. Each band of Bunger represents clusters of Schwann cells enclosed within a common basal lamina (Fig. 1 and Fig. 10). Growth cones in the distal stump become situated between the basal lamina and Schwann cell plasma membrane (Fig. 10). This is in part because

of their affinity for laminin, which is a major constituent of the Schwann cell's basal lamina. The Schwann cell plasma membrane also has growth-promoting, cell adhesion, and recognition molecules. In general, the distal stump shows dramatic changes in gene expression involving an upregulation of neurotrophic factors, neural adhesion molecules, cytokines, and other factors and their related receptors. Many of these responses have been shown to be related to Schwann cells. However, the favorable substrate that Schwann cells establish does not last indefinitely in the absence of axons. The bands of Bunger will ultimately decrease in number when there is limited or protracted regeneration into the distal stump due to extensive scarring at the lesion or long gaps between separated nerve ends. Thereafter, the distal nerve stumps become increasingly collagenous and lose many growth-promoting qualities. In such cases, delayed neuronal death or atrophy can ensue.

Another important cellular response to nerve injury is inflammation and associated removal of cellular debris. The rate at which this occurs can influence the extent of regeneration. In the PNS, myelin debris resulting from Wallerian degeneration is scavenged primarily by activated macrophages that home-in on the degenerating nerve stump within 2 to 3 days after injury. In addition to debris removal, macrophages play other significant roles in wound healing by producing growth factors and certain cytokines with neural growth-promoting properties. For these and other reasons, macrophage responses during Wallerian degeneration in the PNS have been implicated in tissue remodeling, differentiation of Schwann cells, and the establishment of an environment that can support axonal regrowth.

As distal regeneration progresses, Schwann cells begin to remyelinate axons in a manner analogous to peripheral myelination during development, and there is a progressive increase in the number of myelinated axons in the distal stump (Fig. 11). Because of the initial exuberant proximal sprouting response noted above, the number of axons found distally often exceeds the number of parent axons in the proximal nerve stump.

There are other functionally relevant morphologic differences in regenerated versus normal axons. In contrast with intact, adult axons, the myelin internodes (e.g., myelinated segments between successive nodes of Ranvier) are shorter and undergo persistent remodeling for a year or more. In addition, regenerated axons are usually of much smaller diameter with thinner myelin sheaths than that of the parent axon and may persist as such

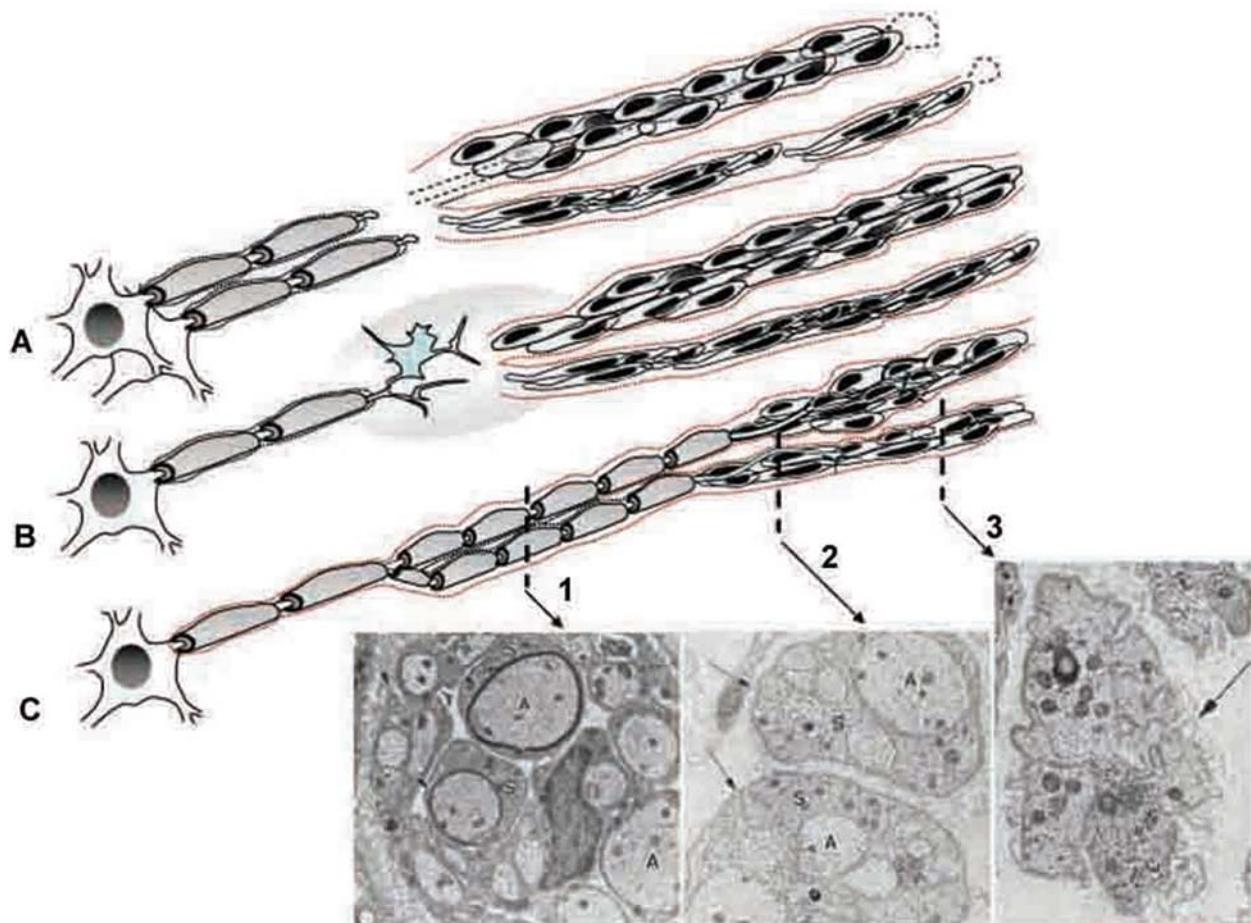


Fig. 10. Three stages of peripheral nerve regeneration are illustrated. **(A)** Wallerian degeneration is completed leaving proliferating Schwann cells that are organized linearly within a common basal lamina (indicated as a sheath around accumulated cells). **(B)** Early regeneration is indicated by the emergence of growth cones from the proximal nerve stump. **(C)** At a more advanced stage of regeneration, axons at a level closest to the injury site (**level 1**) are remyelinated, but their caliber and internodal lengths are less than what they were prior to axotomy. Further distally (**level 2**), regenerated axons are still unmyelinated and closely associated with Schwann cell tubes within a common basal lamina. Regenerating axons may sprout collaterals, and thus the number of regenerated axons may appear greater than axonal numbers in the proximal stump. At a more distal region (**level 3**), prior to growth cone arrival, only Schwann cell processes are seen within the basal lamina. Corresponding electron micrographs are provided to further highlight these features. (Electron micrographs have been adapted for illustrative purposes with publisher permission from Figures 10 to 12 of Osawa et al., Allogeneic nerve grafts in the rat, with special reference to the role of Schwann cell basal laminae in nerve regeneration. *J. Neurocytol.* 1990;19:833–849.)

throughout the course of regeneration. Consequently, regenerated nerves can exhibit markedly altered conduction properties during the course of regeneration.

3.4.2. FUNCTIONAL RECOVERY AFTER PERIPHERAL NERVE INJURY IS DEPENDENT ON THE RATE OF AXONAL ELONGATION AND DISTANCE OF THE LESION FROM THE ORIGINAL TARGETS

The total time for regeneration to be completed is based on (a) an initial proximal healing time and die-back, (b) the time it takes the earliest growth cones to

navigate through the lesion and enter the distal stump, and (c) the length that the distal stump regenerating axons have to grow to reach a target. A related consideration is that not all cells regenerate at the same time. The rate of axonal regeneration is influenced by a variety of factors including the onset of regrowth, age, the location and type of injury, and the period that the distal stump is denervated before the first arrival of regenerating fibers. As a rule, nerve regeneration progresses 3 to 4 mm/day after crush and approximately 2 to 3 mm/day after transection injury. In humans, it has been commonly held that axonal regrowth proceeds at a relatively

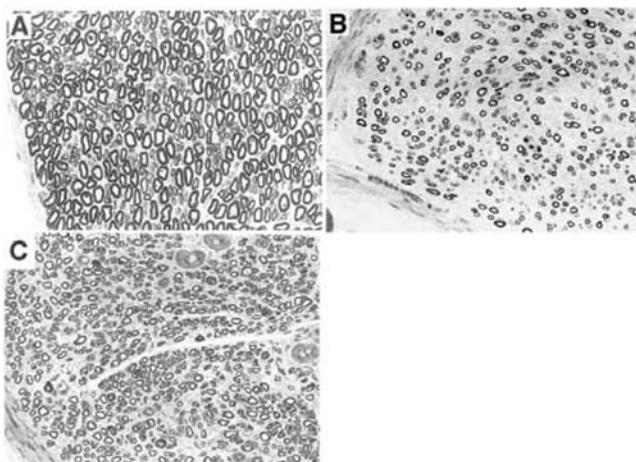


Fig. 11. (A) A cross section of a normal peripheral nerve in which many myelinated profiles are seen. (B) An intermediate time-point during regeneration of comparable nerve. Note the initial difference in the number of myelinated fibers and their smaller diameters. (C) At a more advanced stage of regeneration, whereas the number of myelinated axons is much greater, their diameters remain smaller than normal.

constant rate of 1 mm/day or 1 inch/month, but it has been shown that this rate diminishes over time. Given the relatively slow rate of regeneration, persistence of viable targets becomes highly dependent upon how far regenerating axons must grow. As noted previously, denervated muscles can become atrophic because of the loss of neurotrophic support and functional disuse (Fig. 6). Therefore, if the injury is a considerable distance from the target, then even under optimal conditions, the chance of reinnervating viable muscle becomes increasingly remote.

Although distal Schwann cell tubes provide a compatible cellular highway for axonal regrowth, this matrix does not guarantee that fibers will reach appropriate target sites. Even at a macroscopic level, the characteristic multifascicular nature of a normal peripheral nerve is readily apparent (Fig. 12A). From a three-dimensional perspective, these fascicles establish a complex interconnecting landscape (Fig. 12B), and any disruption of the continuity of this geometry by injury can readily cause regenerating axons to become misdirected as they enter the distal stump. For that reason, the potentially different functional outcomes of crush versus transection injuries become more understandable. A nerve crush yields the most favorable setting for unimpeded axonal regeneration and end organ reinnervation because the integrity of surrounding epi- and perineurial connective tissue sheaths of the nerve is preserved. Moreover, after

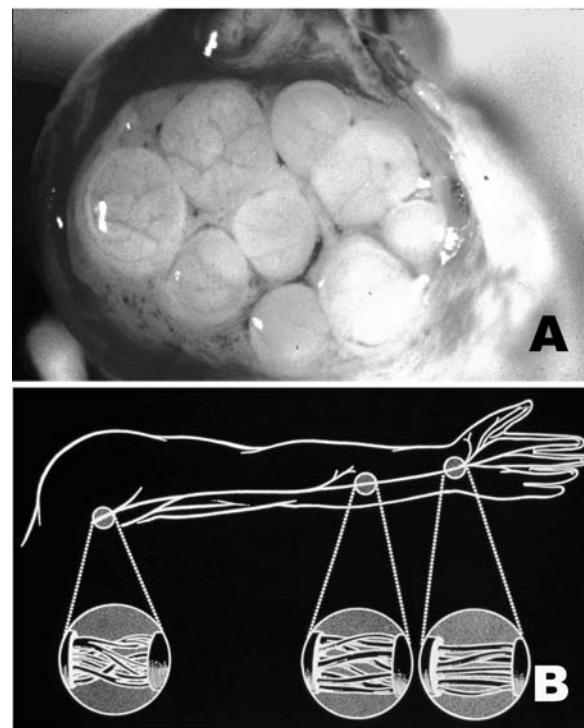


Fig. 12. The complex three-dimensional geometry of a peripheral nerve is shown macroscopically (A) and as a diagrammatic reconstruction (the medial nerve of the arm, B). See text for details. (Illustration provided by Dr. Susan E. Mackinnon, Washington University School of Medicine and Barnes-Jewish Hospital.)

nerve compression, endoneurial fascicular geometry is not radically perturbed, thereby minimizing scar tissue in the lesion site and preserving the internal architecture of the nerve.

In cases of nerve transection, one surgical procedure involving short lesion gaps is to suture the proximal and distal nerve ends together in such a way that opposing proximal and distal perineurial fascicles are matched as much as possible. Unfortunately, even this approach has its limitations due to scarring at the site of repair and poor matching of the fascicles. For this reason, the closer the lesion is to its original sites of innervation, the greater the probability of achieving appropriately directed growth to correct targets.

Therefore, the success of PNS regeneration, though significantly influenced by neuronal metabolic vigor, ultimately becomes even more dependent upon appropriate axonal guidance. Misrouting of regenerating axons is a common feature of peripheral nerve injury, and functional outcomes can be mixed. Poor recovery will occur when regenerated sensory fibers establish a pattern of reinnervation unlike their normal distribution within a given dermatome.

Likewise, regenerated motor axons from flexor motoneuron pools, for example, may end up reinnervating extensor muscles. Thus, improving the accuracy of PNS regeneration remains a significant challenge in peripheral nerve repair.

3.4.3. PERIPHERAL NERVE INJURIES AND REARRANGEMENT OF NEURAL CIRCUITRIES IN THE CNS

Appropriate targeting of regenerating axons is critical to the reestablishment of proper functional circuit domains in the spinal cord and brain. CNS changes in response to peripheral axotomy represent one example of *neuroplasticity*, which will be discussed in more detail in Section 7.2. For the current discussion, it should be mainly noted that numerous studies have demonstrated that injured neurons respond by retracting their dendritic trees, thereby resulting in a depletion of synaptic inputs. In the case of motoneurons, synaptic detachment is a consistent aftermath of peripheral nerve injury and seems associated with astroglial and microglial reactivity in spinal cord gray matter. Additional changes in synaptic input and efficacy among other spinal, subcortical, and cortical neuronal responses (e.g., pharmacologic) follow and may be rapid in onset.

The duration such changes are maintained depends upon the speed and accuracy of regeneration. In the permanent absence of target reinnervation, an extensive body of literature shows that peripheral nerve lesions with regenerative axonal misrouting and target mismatches can be accompanied by dramatic reorganization of CNS neural circuitries. These can range from changes in the projection patterns of spinal primary afferents to alterations in somatosensory maps at cortical levels. Such errors can have profound influences on functions, which often become maladaptive (as in the case of somatosensory reorganization and phantom pain often exhibited by amputees).

3.5. Summary of Postaxotomy Responses in the PNS

This general review of peripheral nerve axonal degeneration and regeneration has highlighted several basic aspects of nerve injury and regrowth that will serve as a basis for later discussions of regeneration in the injured CNS. As shown in Table 2, the essential prerequisites for optimal regeneration fall under two main headings: intrinsic and extrinsic regulation. The former refers to properties inherent to the neuron itself, whereas the latter addresses molecular and cellular influences from the environment surrounding

Table 2
Basic Requirements for Optimal Nerve Regeneration

Intrinsic or Native Properties of the Neuron

- Neurons that have been injured must have the metabolic capacity to initiate and maintain regrowth of damaged axons over long distances.

Extrinsic Conditions

- Regenerating axons should be able to encounter a surrounding cellular environment that is compatible (actively or passively) with fiber outgrowth.
- Ideally, the cellular terrain should provide cues (chemical, physiologic, etc.) that can help guide axons to their correct targets.
- Regenerating axons should be able to respond to these cues and ultimately establish functional relationships with appropriate targets.

the injured neuron. Under optimal conditions, regeneration in the PNS holds promise for useful functional outcomes because neurons can often muster sufficient metabolic capacity to initiate and sustain active regrowth through various alterations in neuronal gene expression. Furthermore, the growth cones of the newly formed neurites are exposed to a cellular and molecular milieu that, in the absence of severe scar formation and other limiting factors, is favorably disposed to extensive axonal elongation. However, the speed of regrowth and successful targeting represent major limitations to optimal functional returns in most cases. Failure to reestablish appropriate innervation can also result in long-lasting circuitry changes (anatomic and functional) at spinal and supraspinal levels.

4. NEURONAL RESPONSES TO INJURY OR DISEASE IN THE CENTRAL NERVOUS SYSTEM

Extensive neuronal cell death can occur within the CNS as a result of neurodegenerative disease, normal aging, infections, tumors, or trauma. Many advances have been made in identifying mechanisms associated with different forms of neuronal loss, such as described above, and it is now recognized there are a number of initiators of cell death in the nervous system. The cellular responses of neural tissue to traumatic, metabolic, or viral insults, to name a few, can vary and depend on many conditions. However, despite the variety of triggers and their modulation by genetic and epigenetic factors, the subsequent molecular processes that result in cell death are highly conserved and often similar to mechanisms of cell death in other tissues.

4.1. Primary and Secondary Cellular Responses to CNS Injury

Cells in the CNS can be damaged directly or indirectly. In the majority of cases, direct damage to the CNS initiates forces that cause vascular rupture (*see later*) and devastating, immediate physical insult to neurons, white matter tracts, and glial cells. The immediate, collective outcome of this mechanical disruption is irreversible tissue destruction and cell death, referred to as *primary injury* or *tissue necrosis*. In contrast, another form of cell death is *apoptosis*, or *programmed cell death* (PCD). This is a process by which a cell undergoes a genetically sequenced form of cellular suicide that is also seen during neural development and in other organ systems throughout life. Apoptosis involves a transition from an intact, metabolically active state to cellular breakdown as a result of highly orchestrated biochemical reactions that cleave structural and nuclear proteins in an orderly, stepwise fashion. Apoptosis is believed to be the main form of cell death that in broader terms contributes to the phenomenon of *secondary* or *bystander* (i.e., collateral) *tissue damage* whereby cell populations that do not sustain direct injury nonetheless become vulnerable to a later cell death. *Secondary tissue injury*, which can evolve over a period of hours or weeks after primary tissue damage, results in exacerbated tissue deterioration (Fig. 13).

Both neurons and glia can undergo apoptosis. For example, experimental spinal cord lesions can entail an initial and a delayed wave of oligodendroglial cell death in white matter tracts both at and distant from the site of injury. Because oligodendrocytes produce myelin, loss of these cells without concomitant degeneration of associated axons (i.e., which are spared because they escape primary or secondary injury) can result in demyelination such as exhibited in multiple sclerosis plaques. Affected axons will have severe deficits in action potential propagation that accounts for some functional deficits after injury.

4.2. Formation of Reactive Oxygen Species (i.e., Free Radicals) and Secondary Tissue Injury

The underlying principle of secondary injury is that damaging agents or biochemical mediators are released after the original insult, and these byproducts participate in other destructive reactions that contribute to further tissue demise. Although the biochemical cascades underlying secondary tissue damage are

very complex, they can be reduced to two fundamental principles: *oxidative stress* and *excitotoxicity*.

Paradoxically, cellular energy production normally leads to the formation of *reactive oxygen species* (ROS) that are highly toxic as they contain one or more unpaired electrons and are, therefore, potent oxidizers of proteins, lipids, and DNA. Several antioxidant detoxification systems have evolved in mammals to offset the toxicity of ROS. These include enzymes such as catalase, glutathione peroxidase, superoxide dismutase, and antioxidants such as vitamins E and C. Because of its high lipid content and because gray matter has a higher metabolic rate than that of most tissues, the nervous system is especially susceptible to oxidative stress. For that reason, either injury or neurodegenerative disease states in themselves can compromise antioxidant defenses by reducing the number of viable neural cells that are available to produce antioxidants and by depleting the intracellular pool of antioxidants in individual cells. Consequently, a greater number of ROS are generated in the nervous system relative to its size in comparison with other organs. Therefore, the balance between pro-oxidant and antioxidant species must be maintained for cellular health. Under many pathologic conditions that give rise to tissue oxidative stress, this balance is perturbed thereby resulting in membrane lipid peroxidation—a destructive reaction that ultimately leads to the formation of lipid peroxides within cell membranes and organelles. Membrane destruction ensues and is accompanied by the loss of membrane-bound ionic pumps, transporters, and receptors. Furthermore, ROS can destroy the structural integrity of protein and DNA molecules in all areas of the cell, thereby disrupting enzyme-mediated catalytic reactions and DNA repair and replication. Thus, oxidative stress can have adverse impact on virtually every cellular function. In addition, membrane lipid peroxidation may also occur in endothelial cells that form the vessels supplying the nervous system, resulting in vascular compromise (*see Section 4.4*).

4.3. Excitatory Neurotransmitters Also Contribute to Secondary Tissue Damage

Trauma and certain neurodegenerative diseases can also induce widespread and massive neuronal depolarization leading to an excessive release of excitatory amino acid (EAA) neurotransmitters, primarily glutamate and aspartate. This situation, which becomes highly cytotoxic, is further exacerbated by disruption of glutamate reuptake. EAA-associated secondary neuronal death, known as *excitotoxicity*,

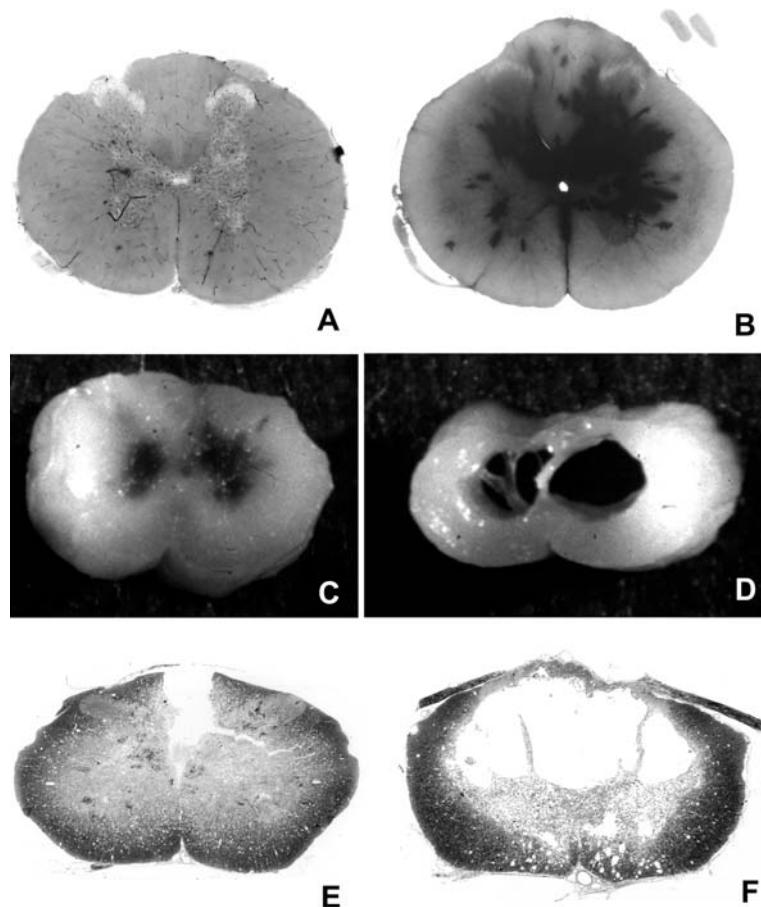


Fig. 13. (A) Blood vessel distributions are shown in the normal rat spinal cord by vascular filling with a tracer. Note that the tracer is confined to the vasculature, which is most dense in gray versus white matter. (B–F) Examples of primary and secondary tissue damage in different tissue specimens obtained at acute and chronic intervals after similar experimental spinal cord injuries in the rat. (B) Three hours after a clinically relevant contusion injury, vascular damage results in extensive extravasation of tracer, which is seen predominately in the gray matter. Blood vessels in the surrounding white matter become relatively constricted. (C) A macro tissue slice of a rat spinal cord obtained shortly after a comparable contusion injury. Note the extensive hemorrhaging in central gray matter as seen in (B), which is due to vascular damage and associated with initial neural tissue necrosis. (D) Two months after injury, tissue loss can be observed extending beyond the initial area of necrosis. The hemorrhaging seen within central gray matter in sections (B) and (C) contributes to progressive tissue degeneration resulting in the development of large cystic cavities. (E) At the histologic level, acute tissue damage as seen in (B) and (C) is characterized by a near total loss of central gray matter definition, with exception of a persistence in this case of the superficial dorsal horns. Within the highly edematous gray matter, darkly stained clusters of blood cells can be seen due to extensive bleeding. (F) By 2 months after injury, tissue degeneration is advanced, and regions of gray and white matter loss are represented by the development of cystic cavities.

follows because of excessive activation of EAA ionotropic and metabotropic receptors with resulting malfunction of Ca^{2+} and Na^+/K^+ -ATPase ionic pumps. Consequently, major alterations occur in extra- and intracellular gradients of sodium, potassium, and other ions. These ionic shifts are followed by massive edema at the injury site causing cellular swelling, expansion of the potential extracellular space, loss of the vascular functional integrity, and increased blood-CNS barrier permeability (see Section 4.4).

Although sodium and potassium fluxes mediate cell death directly by disruption of intracellular and extracellular fluid and electrolyte balance, increased and unregulated intracellular calcium also has profoundly injurious effects. It induces phospholipases that affect the integrity and physiology of cellular membranes and protein kinases that alter signaling and gene expression. Apoptosis-inducing proteases and endonucleases are calcium-sensitive, and toxic intracellular levels of calcium also promote the

activity of some proteins, such as calpain, which cleave cellular structural proteins and thus degrade the neuronal cytoskeleton and disrupt axoplasmic transport. Increased intracellular calcium also leads to elevated production of damaging ROS. Thus, excitotoxicity and free radical formation are not mutually exclusive mechanisms of secondary tissue damage (Fig. 14). For such reasons, disruption of calcium homeostasis is believed to be the most deleterious pathophysiologic event of secondary injury.

4.4. Vascular Disruption and Secondary Tissue Damage

Secondary cell death is frequently associated with concomitant vascular damage and vasogenic edema. Edema can result from many types of insult such as trauma, stroke, neoplastic growths, or abscesses. As noted above, cytotoxic swelling of neurons and glia is a frequent hallmark of edema due to altered ionic

gradients resulting in an unregulated cellular influx of water, cellular osmotic expansion, and subsequent cell death. Vascular damage can also give rise to tissue ischemia, which contributes to cell death and triggers a cascade of secondary events leading to excitotoxicity. Edema may then cause compression of tissue and surrounding vessels, contributing to an exacerbated ischemic response followed by an increase in free radical production and oxidative stress.

Vascular disruption also compromises the blood-CNS barrier, and it has been shown experimentally that the influx of plasma proteins into CNS tissue can be detrimental to neuronal viability. For example, the release of heme due to bleeding further contributes to the production of ROS and membrane lipid peroxidation not only in surrounding neural tissue but also in endothelial cells themselves. Loss of endothelial cell integrity further augments blood-CNS barrier disruption and extravasation.

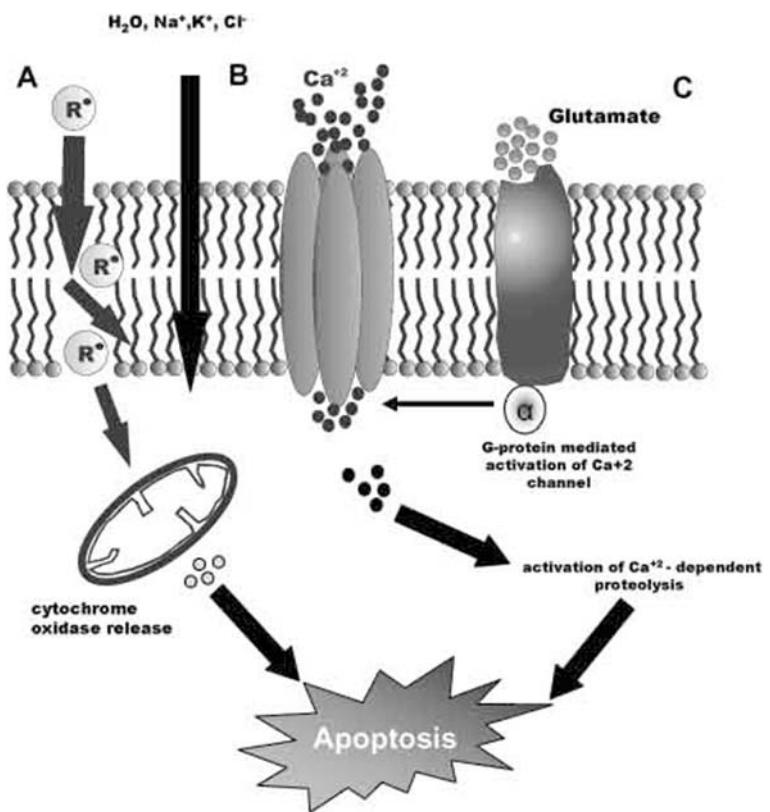


Fig. 14. Secondary tissue damage involves a large constellation of biochemical and molecular events that basically focus on two converging primary mechanisms: lipid peroxidation and excitotoxicity. (A) The formation of reactive oxygen species (or free radicals, R^{\bullet}) precipitates deterioration in membrane integrity with (B) resulting in abnormal influx of water and ions. Free radicals can also affect mitochondrial function leading to increased release of cytochrome oxidase and progressive loss of cellular viability via intracellular apoptotic pathways. (C) Meanwhile, excessive release of excitatory neurotransmitter can lead via ionotropic (not illustrated) or metabotropic receptor activation to increased influx of Ca^{2+} and subsequent activation of proteolytic mechanisms and ultimately cell death via the same apoptotic pathways stimulated by free radical damage.

CNS barrier disruption not only occurs when vessels are compromised by trauma, but can also arise in inflammatory states associated with CNS disease. Experiments have shown that local or systemic inflammation can alter the permeability of the blood-brain barrier to permit plasma protein entry into the CNS. Furthermore, when inflammatory reactions occur such as after CNS trauma, disruption of the blood-CNS barrier is biphasic. In the first phase, disruption of the barrier occurs immediately, and impermeability to large molecules (e.g., albumin) is usually restored within a few hours. The second phase, which arises due to an increase in inflammation, usually occurs days after the initial injury.

5. CNS NEURONS CAN EXPRESS CONSIDERABLE INTRINSIC GROWTH CAPACITIES UNDER CERTAIN CONDITIONS

As in the case of peripheral nerve injury, CNS trauma and certain disease conditions entail axotomy and subsequent Wallerian degeneration (Fig. 2). Whereas in the PNS axonal regrowth can be robust, contrasting circumstances exist in the CNS, which has been scientifically recognized for more than two centuries. In the late 1800 s, the German scientist H. Stroebel demonstrated that after spinal cord injury in the adult rabbit, severed fibers began to regrow. However, they failed to fully regenerate, and restitution of fiber tracts in the spinal cord did not occur. This was later verified by the renowned Spanish neuroscientist Santiago Ramon y Cajal whose research established that nerve fibers in the adult mammalian CNS show an initial outgrowth after injury that may extend at best for approximately 0.5 mm. Thereafter, regeneration comes to a halt, and the newly formed sprouts die back or become arrested, stationary terminal enlargements (a.k.a. retraction bulbs). This transient regrowth of axons within the CNS has led to the long-held view of *abortive regeneration*. It has been inferred from this that most neurons confined to the brain, spinal cord, and retina lack the intrinsic metabolic ability to sustain long-distance regeneration. This view anchored clinical and basic science thinking, both of which maintained that CNS injuries were totally irreversible.

New perspectives began to evolve, however, in the early 1980 s when electron microscope studies revealed indications of synaptic reorganization in the injured adult brain. In subsequent landmark studies, evidence was obtained that precipitated a major restructuring of basic scientific and clinical

views on the potential for regeneration in the CNS. In one set of experiments, investigators introduced one end of an isolated peripheral nerve segment into the midthoracic spinal cord region of a rat and inserted the other end of the nerve graft into the medulla (Fig. 15A). An axon-free bridge (recall that Wallerian degeneration occurs in this preparation), consisting of Schwann cells and other peripheral nerve tissue elements (refer also to Fig. 1), was thus made that extended several millimeters from end to end. Subsequent neuroanatomic evaluations showed that neurons intrinsic to the CNS could extend fibers into these bridges and traverse the entire length of the grafts. More recently, grafts of purified Schwann cells (Fig. 15B) have been made with comparable results,

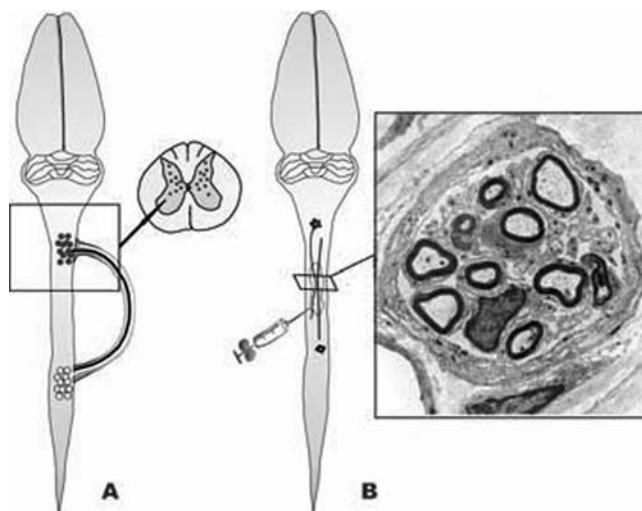


Fig. 15. Two examples of peripheral (PNS) to central nervous system (CNS) grafting experiments that have illustrated the intrinsic capacity of CNS neurons to initiate and sustain axonal growth over substantial distances when the CNS cellular microenvironment is replaced by that of the PNS. **(A)** A peripheral nerve bridge formed between the lower medulla/upper cervical spinal cord and upper lumbar spinal cord. Neuroanatomic tracing subsequently showed axons extending through such grafts. Neurons from which the axons in peripheral nerve grafts arose were demonstrated by a retrograde tracing method (*filled and unfilled circles*), and their distribution in spinal gray matter is illustrated by the accompanying transverse section of the spinal cord (*arrow*). **(B)** Schwann cells that were grown in tissue culture are injected into the rat spinal cord, where they form an intraspinal SC bridge in the completely transected, adult rat thoracic cord. Several weeks after transplantation, numerous fibers have regenerated into the bridge as seen in the accompanying electron micrograph. Such profiles are very similar to what is seen during peripheral-nerve regeneration. (The electron micrograph was kindly provided by Drs. Henglin Yan and Mary Bartlett Bunge, The Miami Project to Cure Paralysis, University of Miami School of Medicine.)

thereby further underscoring the important roles Schwann cells play in promoting axonal growth not only in the PNS but also in the CNS. In some cases, the elongation of axons exceeded the distances originally exhibited by comparable neuronal populations during development. In addition to revealing such remarkable growth potential, other studies have indicated that certain functional synaptic connections can be reestablished in the mature CNS through peripheral and other types of favorable graft tissue (see Section 8.3). Therefore, some degree of appropriate target recognition and functional reinnervation can occur, even in the mature CNS, when regeneration is induced.

6. A BALANCE BETWEEN INTRINSIC AND EXTRINSIC FACTORS GOVERNING AXONAL REGENERATION IN THE CNS

Although CNS neurons have more intrinsic growth potential than previously recognized, it is equally apparent that a need exists for finding ways to augment that capacity. This is especially underscored by the fact that the PNS-to-CNS nerve grafting experiments also revealed how unfavorable the CNS milieu can be to regeneration. When centrally derived axons growing through PNS grafts exited at the opposite end, they invariably stopped elongating once their growth cones reestablished contact with CNS tissue. Therefore, an important corollary to the demonstration of regenerative potential in the injured CNS with PNS grafts, as described above, is that such a capacity can only be seen when the CNS cellular environment is replaced by a cellular microenvironment that is permissive to axonal elongation.

For many years, researchers held the notion that a major limitation to regeneration in the CNS was the absence of Schwann-like cells that could facilitate axonal regrowth via a permissive milieu involving the elaboration of neurotrophic and neurotropic factors. In addition, several other nonpermissive cellular/molecular environmental conditions have now been identified that currently center mostly on the inhibitory effects of astroglial scars and CNS white matter (e.g. Table 1). Inadequate inflammatory responses, coupled with protracted debris removal, also have been implicated.

As a prelude to the following discussion, it should be emphasized that the problem of axonal regenerative failure in the CNS is a complex one, and there are no absolute rules. Accordingly, the lack of spontaneous axonal regrowth is not exclusively due to the

presence of any one or more nonpermissive tissue environments; it also can be coupled with modest growth responses exhibited by different populations of injured neuron. In fact, several studies have shown that the administration of certain neurotrophic factors can significantly enhance the vigor of axonal elongation in the presence of cellular environments, which otherwise would be major obstacles.

6.1. Astroglial Environments Can Impede Axonal Outgrowth

In cases of neurotrauma and disease, the loss of neurons and/or their processes triggers an astroglial response in which those glial cells increase in cell size, as well as in the thickness and number of their cytoplasmic processes. This overall astrocytic response is referred to as *gliosis* or *astrogliosis*, which in conjunction with other cells can form *glial scars*. In many ways, astroglial scarring is a form of wound healing, and experiments have shown that if the CNS is rendered deficient in astrocytes, the degree of secondary tissue damage is much greater. Although in many respects gliosis may be a way to restore physiologic homeostasis within the CNS, it also has a downside as considerable evidence exists showing that in conjunction with other cells, glial scarring represents a significant impediment to regeneration.

In the case of laceration injuries causing the interior of the CNS to be breached (e.g., by stab wound, gunshot, or bone fragments entering the CNS), astrocytes respond by reinstating a glial limiting membrane along the exposed CNS neural parenchyma apposing non-CNS tissues (e.g., fibroblasts, meninges, and collagen). Eventually, this forms a type of glial capsule (Fig. 16A) or partition (Fig. 16B) that can be one or more layers thick and walls off the interior of CNS tissue from non-CNS elements in the lesion. This form of astroglial activity thus serves to repartition the CNS compartment from non-CNS tissue elements after injury.

Astrocytic reactivity, characterized by hypertrophy of the cell and its processes and varying degrees of cell proliferation, also occurs along damaged white matter tracts and in gray matter as an aftermath of Wallerian degeneration (Fig. 17). In degenerated white matter, a dense astrocytic meshwork is formed over time that most notably consists of enlarged and intertwining hypertrophic astroglial processes, which at the ultrastructural level contain large accumulations of glial intermediate filaments. Similarly, astrocytes exhibit considerable reactivity in regions of gray matter after fiber and axon terminal degeneration

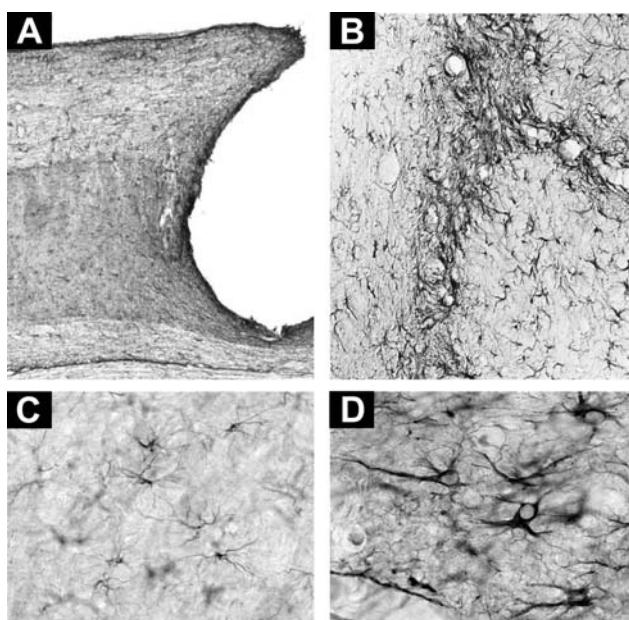


Fig. 16. Astrogli reactivity after CNS injury. Astrocytes are demonstrated in the figures by staining with an astroglial-specific antibody that is directed at the glial fibrillary protein constituent of astrocyte intermediate filaments. **(A)** A horizontal section of a partially transected rat spinal cord showing dense glial reactivity along the gray (GM) and white matter (WM) margins of the lesion (*arrows*). **(B)** Intense glial reactivity is demonstrated in relation to the equivalent of a stab wound in the CNS. Some gliosis is also seen in association with blood vessels (*bv*) at this lesion. **(C)** Astroglia in normal gray matter are shown to compare with **(D)** hypertrophic, reactive counterparts in gray matter distant to a spinal cord lesion.

and in response to primary or secondary neuronal death (Fig. 16C, D), but this usually does not develop into the extensive three-dimensional astroglial scars seen in lesions and in degenerated white matter.

There are several examples of reactive astrocytes having inhibitory effects on growing axons. One involves crushed or cut dorsal-root axons, which, after injury, regenerate and advance vigorously within the peripheral nervous system compartment of an injured spinal sensory root. As they reach the spinal cord dorsal root entry zone (DREZ), these axons terminate either as large bulbous enlargements, assume random growth patterns, or are deflected back toward the spinal ganglion of origin (Fig. 18). The DREZ, also known as the PNS-CNS transition zone, is characterized by a progressive shift from a peripheral to a CNS tissue terrain. The altered axonal growth pattern at this interface appears to be associated with the presence of astrocytic processes that are interspersed with Schwann cells and endoneurial

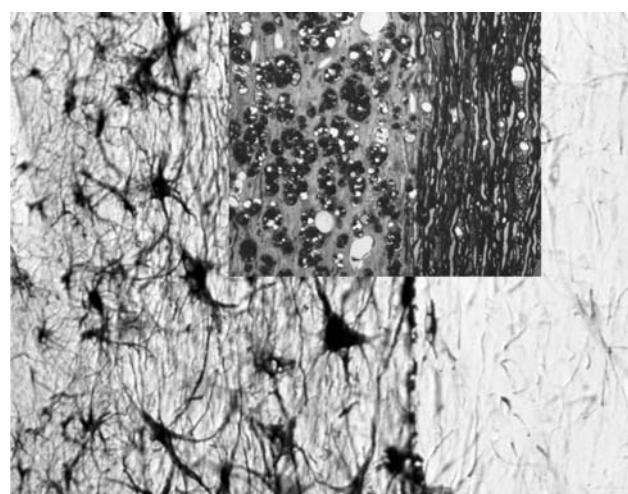


Fig. 17. *Inset:* A longitudinal section of the rat spinal cord after hemisection. Bundles of normal, myelinated axons are seen in white matter on the intact side of the spinal cord (*right*), but on the lesioned side (*left*) advanced degeneration is apparent and is indicated by macrophages and myelin debris (*darkly stained profiles*). The section was obtained caudal to the level of injury. *Large panel:* Astroglial pathology resulting from the same type of surgical preparation is illustrated using an antibody staining technique directed at a cytoskeletal protein constituent of astrocytes. Notice the extensive astrogli reactivity in white matter on the lesioned side of the cord (*right*).

connective tissue (composed of fibroblasts and collagen) that do not become confluent with the CNS tissue domain.

Because of the complex network of enlarged and intertwined cytoplasmic processes that characterize astroglial scars, one is left with the impression that reactive astrocytes form physical obstacles to growing axons (Fig. 17B, 18E). However, specific molecular properties of these cells also seem to have bearing on glial inhibition of axonal elongation. Several well-defined chemorepellant or growth-inhibitory molecules, some of which are major extracellular matrix (ECM) constituents, have been identified as in the CNS (Table 1) with some being produced by reactive astrocytes. Chondroitin sulfate proteoglycans (CSPGs), which are a complex class of macromolecules, represent one area of considerable current interest in this regard in terms of therapeutic targeting as noted later (*see* Section 8.4) for promoting axonal regeneration in the CNS.

6.2. White Matter–Associated Inhibition

Experimental evidence also indicates that among other axonal elongation inhibitors, proteins associated with myelin and myelin-producing oligodendrocytes

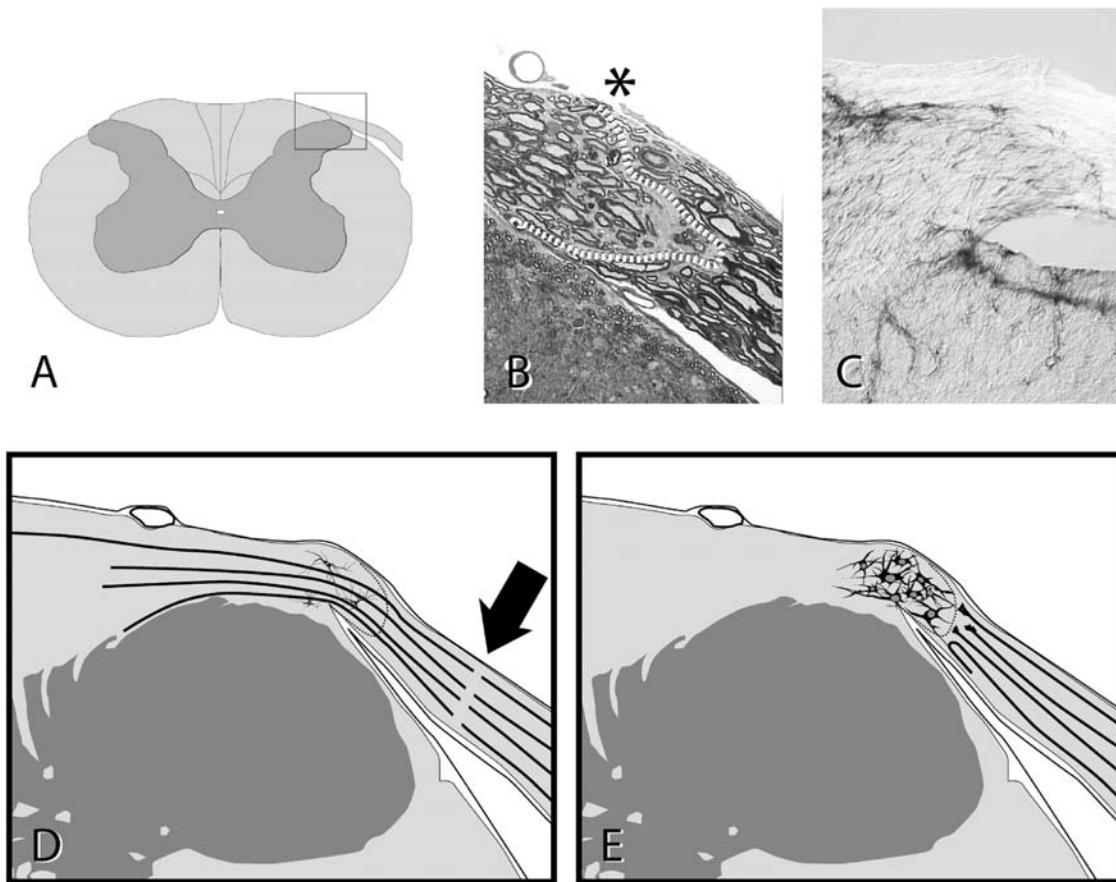


Fig. 18. (A) A schematic diagram showing a cross section of the spinal cord. One experimental model that has demonstrated the growth-inhibitory nature of reactive astrocytes has focused on the dorsal root entry zone (DREZ) or PNS-CNS transition zone of the spinal cord, identified by the boxed region. (B) Histologically, the DREZ (*asterisk*) is characterized by an astrocytic dome-like region (*dashed line*) that demarcates a cytologic shift from PNS cellular constituents of the dorsal root to CNS elements at the point that the central process of dorsal root ganglion cells enter the CNS. (C) Staining for astrocytes, as in Fig. 16, demonstrates the presence of astroglia on the central side of a dorsal root, whereas the peripheral side is negative. Thus, the DREZ identifies with a loss of Schwann cells and the emergence of astrogial and oligodendroglial interactions with primary afferents. (D) When a nerve root is cut or crushed, Wallerian degeneration progresses centrally and triggers astrogliosis at the DREZ. (E) As elsewhere in the PNS, the axotomized sensory cells initiate axonal regeneration; however, once growth cones interact with reactive astrocytes at the DREZ, they either abort their continued advance or are deflected back. The extracellular matrix environment of this region of gliosis has been implicated in the inhibition of centrally directed regeneration.

contribute to growth cone collapse and overall regenerative failure in the CNS (Table 1). It was initially demonstrated that when axons growing in tissue culture encounter a myelin membrane substrate interface, they stop growing or turn away. However, if in the same cultures neurons were given a choice of PNS tissue-coated substrates, their neurites would preferentially extend unimpeded on the PNS surfaces.

One of a group of myelin-associated proteins that can impede axonal outgrowth is referred to as Nogo-A. Receptors for Nogo-A are present on growth cones, and interaction with this receptor results in activation of molecular pathways within the growth cone that

result in depolymerization of cytoskeletal constituents associated with growth cone mobility as well as calcium influx both of which can cause growth cone collapse or arrested movement. Interestingly, if Schwann cells are genetically modified to express Nogo-A, their permissive properties for axonal regeneration are overridden further emphasizing the adverse impact of this myelin-associated inhibitor (MAI). Various experiments have shown that either an antibody directed at Nogo-A or the application of antagonists to the Nogo receptor itself (with which other MAIs also bind) can enhance axonal sprouting and regeneration with associated behavioral improvements.

From a histologic perspective, it is not surprising that some MAIs of axonal outgrowth are also associated with glial scar formation because evolving astrogliotic regions often have large, interspersed accumulations of myelin debris (Fig. 17). It is interesting to note that in the immature mammalian CNS, some axonal regeneration is possible before myelination starts (*see* Section 6.4). As axons become myelinated, their regenerative capacity is reduced. However, by neutralizing the inhibitory myelin-associated proteins, the developmental period for which regeneration occurs can be extended.

6.3. Microglia and Inflammation

Microglia also exhibit reactivity in CNS injury and disease, characterized by altered morphology, increases in cell size and number, and modifications in gene expression (Fig. 19). Microglia are known to have the capacity to synthesize a variety of proinflammatory cytokines that mediate chemotaxis, extravasation, and activation of leukocytes. In that regard, microglia are considered major participants in inflammation and wound healing after CNS injury. They also can transform into brain macrophages (Fig. 19). Astro- and microglial activation usually occurs shortly after axotomy and often well in advance of any overt signs of Wallerian degeneration *per se*. Furthermore, these glial responses are highly restricted. In the case of two adjacent white matter tracts—one damaged, the other intact—only the former will exhibit signs of astro- and microglial reactivity (Fig. 17). This indicates that the signaling mechanisms involved are spatially limited.

Inflammation after CNS injury is a complex problem that under some circumstances can facilitate regeneration whereas under other conditions can be nonpermissive. For example, monocyte recruitment from the blood and macrophage activity in the CNS do not evolve as quickly in the CNS as in the PNS after injury. Combined with other cellular dynamics discussed above, this also may account for limited axonal growth in the injured CNS. There is some indication that such poor cellular recruitment may be related to an absence of induction of the immune complement system as complement attracts and activates macrophages in the PNS. The persistence of myelin-related axonal growth inhibitors and repellent molecules represents a logical result of inefficient debris removal in the injured CNS. In contrast with expedient debris removal distal to a peripheral nerve injury, degeneration profiles and myelin breakdown can be found in the injured CNS for weeks and even a year or more after injury (Fig. 2 and Fig. 17).

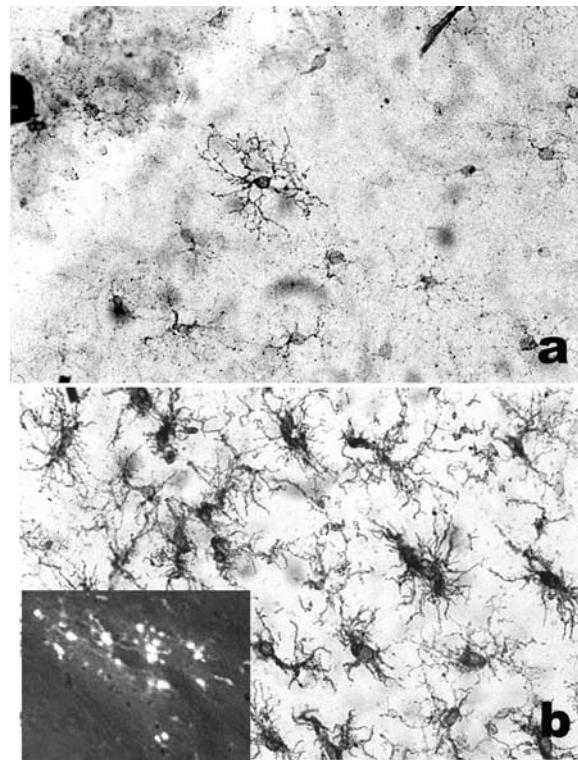


Fig. 19. (a) Resting, ramified (e.g., process-bearing) microglia are shown as seen in the normal CNS. (b) Under most pathologic conditions, these cells increase in number and exhibit hyperplasia and hypertrophy as reactive microglia. *Inset:* Microglia have phagocytosed a retrogradely transported fluorescent label to show apoptosis of axotomized spinocerebellar neurons (*see* Fig. 5). This provides one example of the role of microglia in debris removal after injury or in disease among their many other functions. (Figures A and B were kindly provided by Dr. W.J. Streit, University of Florida, McKnight Brain Institute.)

6.4. Injury in the Immature CNS

It should be noted that the lack of axonal regeneration described so far in the adult CNS contrasts strikingly with numerous experimental reports of regeneration after injury in the immature CNS. One example has come from experimental spinal cord injury studies in the developing opossum. Being a marsupial, the young are born at a very early stage of development, equivalent to embryonic stages of placental mammals (i.e., eutherians). If the spinal cord is completely transected (i.e., no axons are spared) during the first week of life in the opossum model, the injured axons are capable of regeneration. Locomotor function throughout the remainder of life is much better than that seen in animals injured at later ages. If the opossum spinal cord is injured after 1 week of age, the overall extent of repair and recovery is greatly reduced, and no axonal regeneration has been observed. Similar studies in a number of other immature mammalian

species have further underscored this principle, however the age at which regeneration occurs in eutherians is embryonic and substantially more difficult to study.

There are many reasons why axonal regeneration is seen only at very early developmental stages, and these help to clarify reasons for the lack of regeneration in the adult CNS. First, during neural development, the levels of neurotropic factors and guidance molecules are greater than those in the adult CNS. In addition, the degree of myelination (as described above) is less than that seen in adulthood, so the extent of myelin-associated growth inhibition is reduced. Although beyond the scope of this chapter, it should be noted that recent experiments have shown that the timing and extent of cellular and molecular inflammatory responses to injury in the developing CNS differ from that seen in the more mature CNS. In addition, cellular reactivity to spinal cord injury has been shown to be less severe and delayed in the earlier stages of development.

7. THE INJURED CNS HAS SOME INTRINSIC POTENTIAL FOR SELF-REPAIR: CONCEPT OF NEUROPLASTICITY

7.1. Peripheral and Central Axotomy Can Be a Stimulus for Axonal Growth from Uninjured Neurons

There are many examples of self-repair phenomena in the damaged brain and spinal cord that demonstrate a biological potential for spontaneous functional improvements, even after trauma in the PNS (see Section 3.4.3). The mechanism by which this occurs is referred to as *neuroplasticity*, which can be exhibited in a variety of contexts such as anatomic reorganization of neural circuits, neurophysiologic changes, and altered neuropharmacologic dynamics, among other responses to injury or disease. Experiments first performed more than 50 years ago demonstrated the principle of *collateral sprouting* by showing that when some nerve fibers to a muscle are transected, neighboring intact fibers begin to generate accessory axonal branches *de novo* and subsequently innervate the partially deafferented muscle (Fig. 20A). The same principle was later extended to the CNS where denervation of a neuronal target can lead to the sprouting of adjacent fiber systems and the formation of new synapses onto the denervated cell (Fig. 20B). Neuroplasticity offers an explanation for cellular events that can be associated with restoration of relatively appropriate behaviors, the development of compensatory behaviors (adaptive plasticity), or even the emergence of inappropriate

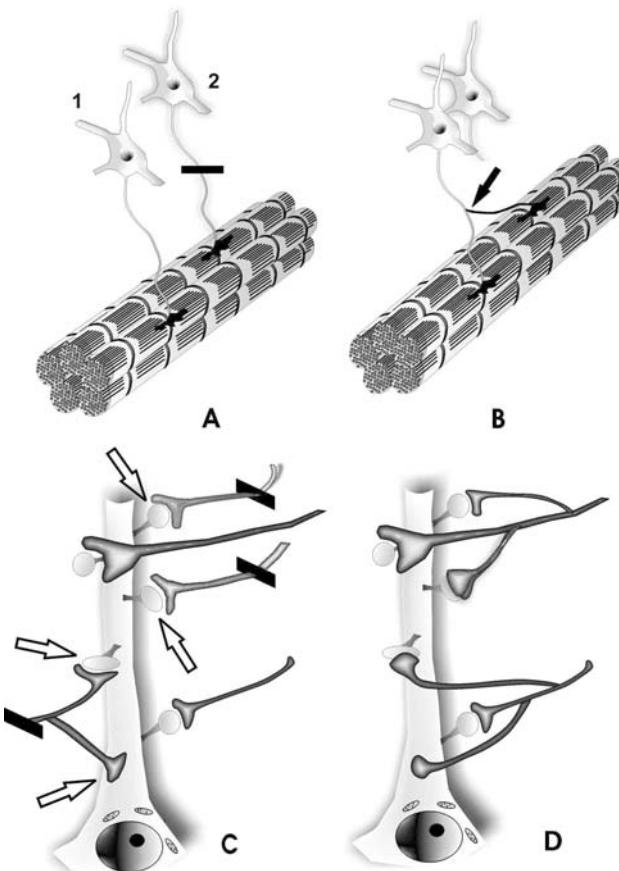


Fig. 20. Collateral or terminal sprouting in the (A) PNS and (B) CNS. Axotomy of a neuron (2) results in the emergence of a new collateral (arrow) from the uninjured cell (1). The new axonal outgrowth reinnervates the neuromuscular junction region originally occupied by the presynaptic terminal of neuron 2. (B) A neuron in the CNS is deafferented due to axotomy (black rectangles) at four sites on either synaptic spines or the shaft of a primary dendrite (open arrows). As in the previous example, short-distance, preterminal collateral sprouting from adjacent, uninjured axons results in reinnervation of the affected neuron.

functions (maladaptive plasticity). Collateral sprouting does not take place over long distances and is generated by previously uninjured cells. Such forms of axonal growth have little significance in terms of regeneration *per se* but offer some perspective on the nervous system's potential for self-repair, which future therapeutic strategies may enhance.

7.2. Functional Compensation or Reorganization Occur in the CNS

Some of the more striking examples of neuroplasticity are derived from neuropathology. Signs and symptoms are often masked for an extended time during disease progression, which suggests adaptation to cell loss. In some instances, collateral sprouting may offset

deneration resulting from neurodegeneration. However, neuroplasticity is not necessarily limited to anatomic modifications and can also be exhibited by pharmacologic and molecular responses, among other possibilities.

Figure 21 depicts another remarkable example of neuroplasticity, seen in this case after spinal cord injury. Shown is a magnetic resonance imaging (MRI) scan of an individual who had sustained spinal trauma at T12, which resulted in severe lower extremity weakness and partial paralysis. This person eventually regained significant ambulatory capabilities with the assistance of bracing and a cane or walker. Approximately 30 years after the initial injury, this individual began experiencing upper-extremity weakness as well as greater ambulatory difficulty. However, there was no evidence of abnormal sensory manifestations such as allodynia, or deteriorated upper-extremity function. Surprisingly, subsequent MRI examination, as illustrated, showed



Fig. 21. A sagittal MRI (*left panel*) is shown of an individual who experienced progressive degeneration of spinal cord tissue over the course of 30 years after an initial spinal injury at T12 (see text for more details). A continuous cystic cavitation (*arrows*) is seen extending to the level of C2. A transverse MRI scan taken at T5 is highlighted to the *right*, showing a large cyst (*dark gray*) and a thin rim of surrounding white matter. Extensive gray matter loss is also suggested at midcervical levels (e.g., region of phrenic motoneurons), however, no respiratory compromise or severe upper-extremity motor abnormalities were noted other than a gradual weakening of strength.

extensive degeneration of the spinal cord from the original level of injury at T12 to C2. This is an example of a slowly progressing secondary degeneration of spinal cord tissue that occurs in a small subset of individuals who previously sustained spinal cord injury. The two examples described illustrate that very devastating, slowly evolving pathologies do not result in the immediate and extensive functional losses that would otherwise accompany such more immediate and large-scale traumatic or neurodegenerative pathologies. This can only be explained by the fact that dramatic levels of neuroanatomic reorganization and functional compensation are simultaneously superimposed on slower degenerative process.

Many instances of plasticity have also been illustrated in studies of human stroke patients and experimental animal models of brain injury. In adult subhuman primates with focal ischemic lesions, it has been shown that subtotal damage restricted to a finite cortical representation of a hand can extend to further loss of such representation in the adjacent, undamaged cortex, as well as changes in other more remote cortical areas. These changes relate to functional/anatomic somatotopic maps, as well as to underlying cellular modifications in the arborizations of dendrites and changes in the distributions of different types of synaptic profiles.

A case of traumatic hand amputation illustrates the concept of neuroplasticity even more dramatically. In this situation, the cortex became reorganized so that areas normally associated with hand function subserved other modalities. This example is illustrated in Figure 22. The type of plasticity described is known as somatotopic remodeling, where in this person's case, regions of the cortex involved with unaffected muscles or skin, and therefore with intact afferents, expanded into the deafferented hand region of the cortex. The region of the cortex controlling the face expanded to include the cortical area previously associated with hand and finger movements. This patient then underwent a hand transplantation procedure. In months after surgery, the new hand gained significant function and the cortical region previously associated with hand function acquired control over the transplanted hands. With time, there was a progressive recovery of the normal somatotopic map.

Although this is an example of CNS reorganization stimulated by massive peripheral tissue trauma, similar cortical dynamics have been reported to follow CNS trauma and various forms of severe hearing and visual loss as well. For example, a case study of an individual who had experienced a high cervical spinal cord injury demonstrated, via functional MRI

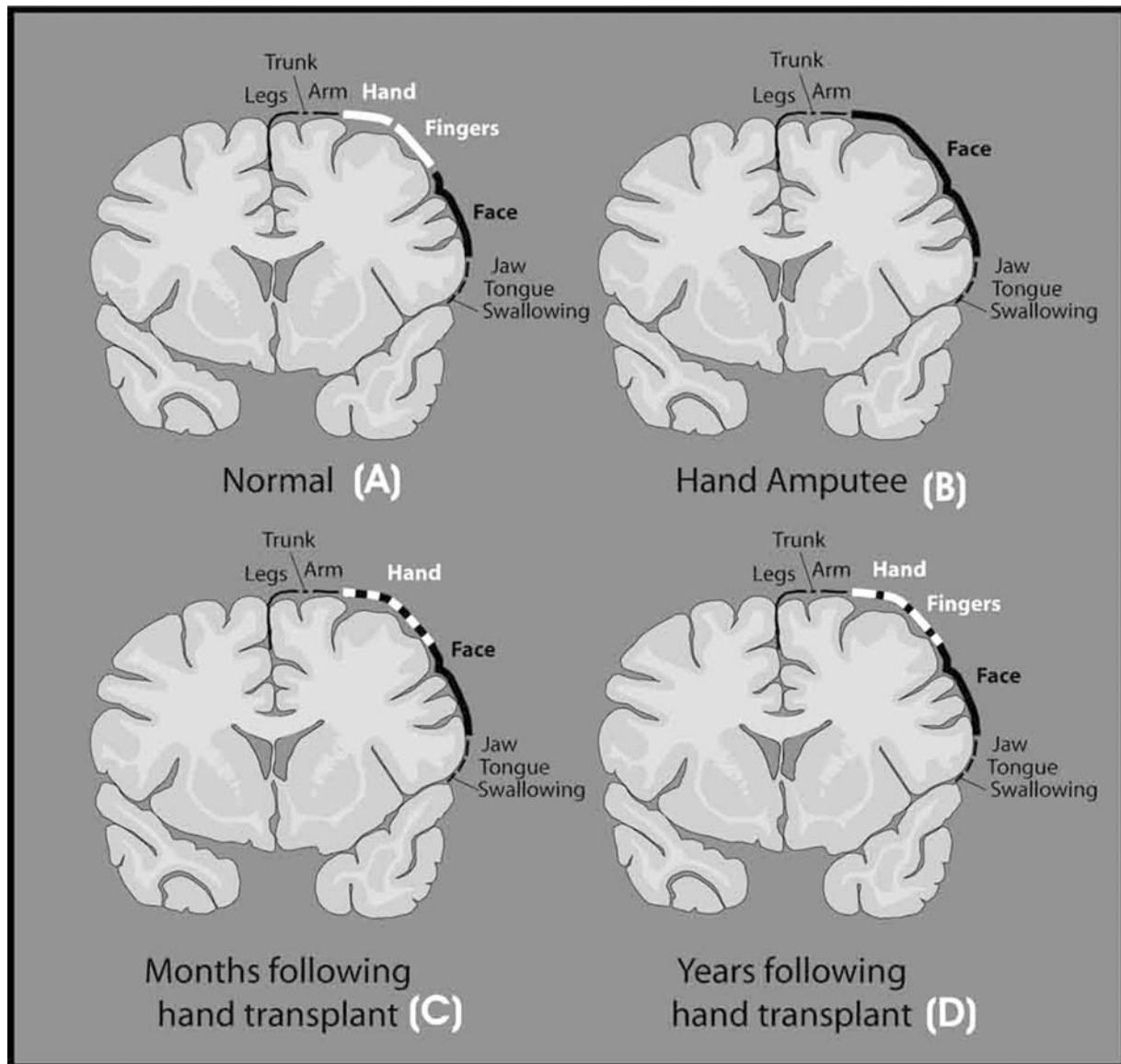


Fig. 22. Cortical reorganization after a hand transplantation procedure is illustrated by this example based on a case report discussed by Farne and colleagues (Farne A et al. Face or hand, not both: perceptual correlates of reafferentation in a former amputee. *Curr. Biol.* 2002;12(15):1342–1346). **(A)** As a point of reference, normal hand representation is shown. **(B)** In contrast, prior to grafting, facial stimulation evokes activity in the former hand region of M1. The perceptual consequences of such reorganization can be detrimental as they can give rise to severe phantom limb pain. Alternatively, touching the ipsilateral face can produce referred non-noxious feelings in the phantom limb. **(C, D)** Months and years after hand transplantation, ipsilateral cortical hand representation is progressively reestablished. Because the procedure also entails peripheral nerve regeneration for innervation of the grafted hand, this type of finding indicates a temporal-orderly and global remodeling of the limb cortical map. However, simultaneously touching the ipsilateral face and the grafted hand can extinguish perception in the new hand.

(fMRI) as above, that vibratory stimulation of a paralyzed hand failed to elicit any response in the hand region of the somatosensory (SI) cortex. Instead, this region was recruited during tongue movements that would be normally associated with more lateral face regions. Ironically, even after a 5-year absence of any function below the shoulders, vibratory stimulation of the foot evoked responses in topographically correct

somatosensory regions. The nervous system thus has not only remarkable potential for adapting to new conditions through change, but it also has the capacity to maintain circuitries for long times even in the absence of movements or ascending sensory feedback. These types of finding have significant implications about predetermined connectivity in the CNS as will be discussed in Section 10.

7.3. Functional Plasticity Can Occur Spontaneously or Be Promoted by Training

Although it is generally believed that cortical neuroplasticity is more vigorously exhibited in the developing nervous system, a large body of evidence also points toward experience- or activity-dependent modifications in receptive-field properties, functional cellular organization and domains, and synaptic efficacy even in the adult CNS. Therefore, the mature nervous system is more mutable than once believed, and functional maps of the motor and sensory cortices are now known to be labile and capable of extensive modifications based on environment and behavioral experiences.

With greater appreciation of cortical neuroplasticity that can be expressed under different circumstances, an important new basis and rationale for neurorehabilitative

medicine has evolved. Other studies of focal ischemic damage in the monkey have also shown that ensuing alterations in the cortical motor map could be prevented by retraining skilled hand use on the affected side. Such findings have suggested that forced use of an affected upper extremity in human stroke subjects may promote motor recovery by modulating neural circuit reorganization in neighboring regions of undamaged cortex or by enhancing functional efficacy of altered circuits. Similar approaches to promote activity-dependent plasticity may ultimately prove useful for cerebral palsy and other brain disorders.

Rehabilitative training approaches are also showing recovery of walking patterns in some individuals who have been partially paralyzed by spinal cord injury (Fig. 23). Initial laboratory studies showed

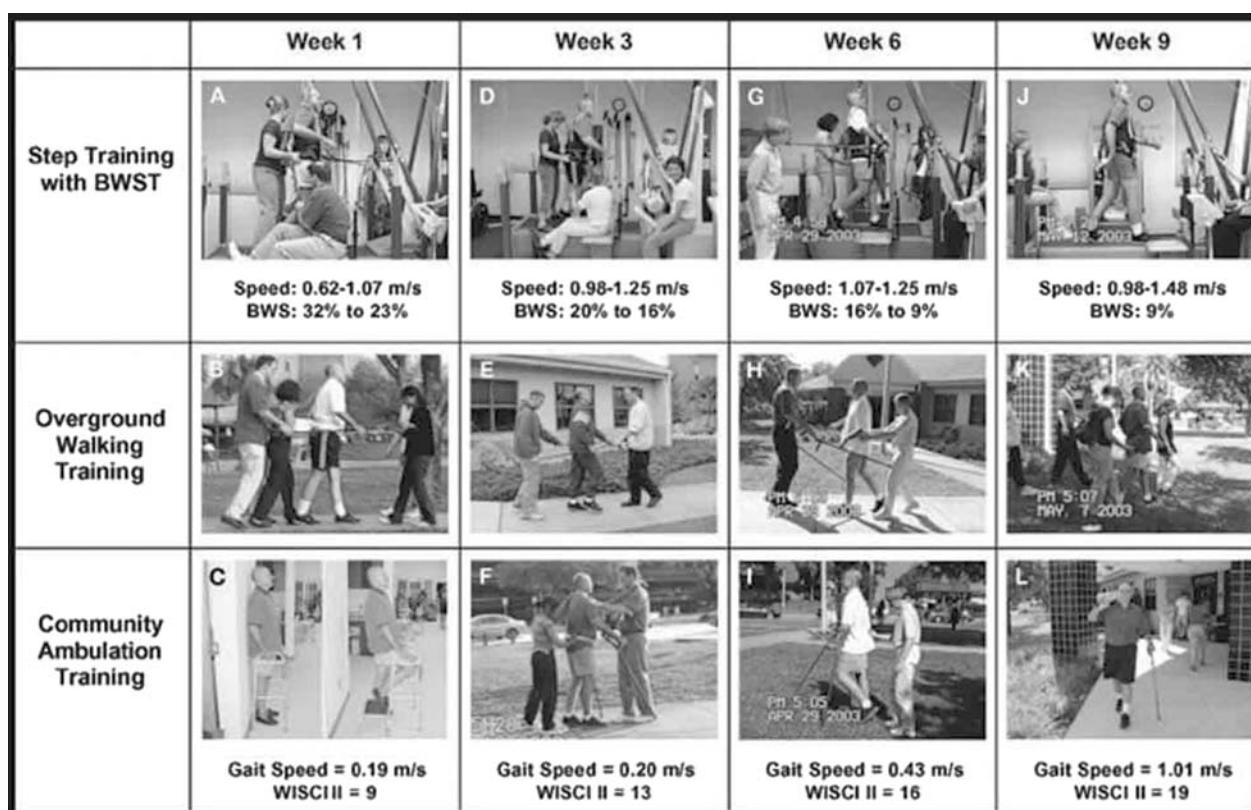


Fig. 23. Studies in recent years have shown that it is possible to restore significant degrees of ambulation after spinal cord injury in individuals who have retained some level of residual motor function (i.e., functionally motor incomplete spinal cord injury). This can be achieved by teaching locomotor circuits in the spinal cord to walk via task-specific, locomotor training using partial body support over a treadmill and manual assistance designed to provide functionally appropriate afferent signals to the spinal cord from the lower extremities. An example of this approach is illustrated by this series of video captures. Initially, the individual shown was trained on a treadmill to relearn stepping. As his gait improved at different treadmill speeds, the extent of body weight support was decreased (*top row*). Skills acquired on the treadmill were assessed daily for translation to overground walking requiring varying amounts of therapist interaction (*middle row*). Ultimately, training progressed to the reacquisition of community ambulation (*bottom row*). (Figure adapted from Behrman AL et al. Locomotor training progression and outcomes after incomplete spinal cord injury. *Phys. Ther.* 2005;85:1356–1371, with permission of the American Physical Therapy Association. This material is copyrighted, and any further reproduction or distribution is prohibited.)

that paralyzed animals could be trained to step on a treadmill when provided with body weight support and repetitive manual assistance. Such recovery is now believed to be due to activity-related sensory inputs to spinal cord circuits involved in alternating limb movements during walking. In that context, these spinal circuits establish a gait pattern and are thus referred to as the *spinal pattern generator*. Such results have now been extended into the clinical setting. Neuroplasticity-based rehabilitation will undoubtedly serve as an adjunct to a broad spectrum of novel therapeutic approaches being investigated for treating brain and spinal cord disease or trauma, as discussed in the following section.

8. POTENTIAL INTERVENTIONS TO PROMOTE CNS REPAIR ARE BASED ON CELLULAR RESPONSES TO INJURY AND DISEASE

To facilitate the regenerative or neuroplastic potential of the CNS, therapeutic strategies must be directed at specific aspects of neuronal injury at the level of the cell body or lesion, in regions of Wallerian degeneration, and at denervated target sites (Fig. 24). Some candidate treatments should promote neuronal survival and stimulate intrinsic genetic programs for the initiation and maintenance of axonal outgrowth, whereas others should render the cellular environment more permissive to axonal elongation. At the same time, reestablishment of connectivity must be facilitated through methods to encourage anatomic and functional plasticity as growth cones advance near prospective target sites and establish new synapses. Finally, to address neuronal cell death, other efforts will be required to replace lost cells.

It is self-evident that the most effective therapeutic approaches will rely on a combination of these treatment modalities. Furthermore, as the previous discussion on neuroplasticity suggests, orchestrated rehabilitative approaches are likely to be at the core of most combined treatments in the future. The following is a brief summary of promising experimental strategies and related technologies for promoting functional repair in the CNS by enhancing neuronal survival, strengthening neuronal regenerative responses, improving the cellular environment at and distal to the site of axotomy, promoting remyelination, and replacing cells that have died. Aside from providing a perspective of possible future advancements in the treatment of major neurologic disorders, the main

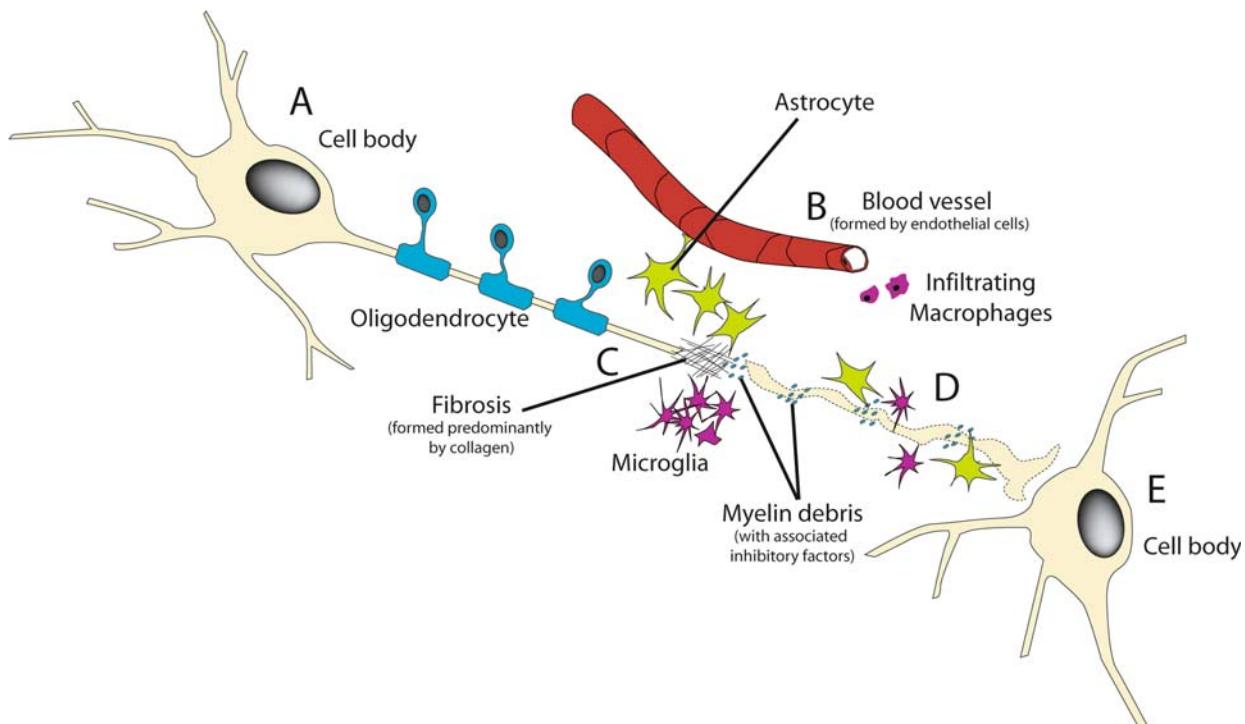
purpose of the remaining discussion is to highlight the important clinical and scientific implications of the basic biology of PNS and CNS injury and disease presented in the preceding overview.

8.1. Injured Neurons at Risk of Atrophy or Imminent Apoptosis Can Be Rescued

Optimization of regeneration and functional outcomes in the CNS is critically dependent upon maximizing the survival of neurons that are at risk of secondary cell death. Ongoing studies are largely investigating pharmacologic *neuroprotective* approaches directed at key biochemical, neurochemical, and molecular mechanisms associated with secondary tissue damage, as previously discussed. An ensuing rescue of cells could therefore translate into less severe neurologic deficits and improved functional outcomes after trauma. Because some neurodegenerative diseases also have been associated with ROS and excitotoxic mechanisms, neuroprotection may prove to be of considerable value in such circumstances as well.

In the case of membrane lipid peroxidation, investigations have shown that agents acting as free radical scavengers or antioxidants could provide some level of neuroprotection. Other promising pharmacologic interventions have been directed at the excitotoxicity component of secondary neuronal responses occurring as part of several neurodegenerative diseases (e.g., Huntington's disease) or as the sequelae to spinal cord or brain injury and stroke. Glutamate pharmacology is one major target, and competitive and noncompetitive glutamate-receptor antagonists have shown therapeutic promise in blocking the binding of excitatory amino acids and reducing cellular toxicity after CNS injury. Enhancement of glutamate reuptake also has been explored with strategies directed at upregulation of glutamate transporters or enhancement of their function. As noted earlier (Section 4.4), excitotoxicity and free radical damage are not mutually exclusive aspects of secondary tissue damage (Fig. 14). One point of cellular and therapeutic convergence could relate to mitochondrial dysfunction after CNS injury. Excessive glutamate release can lead to mitochondrial calcium ion overload and ROS production. Some recent efforts have thus been made to test the neuroprotective efficacy via agents affecting mitochondrial physiology.

Other ways in which neuronal rescue can be achieved involve the use of neurotrophic factors or the activation of antiapoptosis genes. One approach involves the introduction of genes that encode for the production of specific neuroprotective molecules in



EVENTS FOLLOWING INJURY

TREATMENT APPROACHES

Cell body response to axotomy (A) Retrograde cell death Neuronal apoptosis Little or no upregulation of growth-associated genes	At the cell body (A) Pharmacological intervention - e.g. Neuroprotective drugs, Neurotrophic factor delivery Delivery or enhanced in vivo expression of growth promoting and/or cell survival genes Cell replacement strategies
Inflammatory processes Upregulation of inflammatory genes - e.g. cytokines, chemokines Enhanced migration and infiltration of peripheral cells (macrophages, T-cells, neutrophils) Contribution to increased blood-CNS barrier permeability	Inflammatory processes (B-E) Pharmacological intervention to regulate the inflammatory processes Gene delivery to regulate timing and magnitude of inflammation Transplantation of peripheral immune cells (e.g. macrophages)
Vascular compromise (B) Direct vascular damage leading to bleeding and extravasation of circulating immune cells Breakdown of the blood-CNS barrier Increased barrier permeability as a result of inflammatory processes	Vascular compromise (B) Pharmacological interventions to reduce inflammatory response, thereby reducing associated changes in blood-CNS barrier permeability
Cavity and Scar formation at the injury site (C) Accumulation of activated astrocytes and microglia Release of growth inhibitory molecules (e.g. CSPG's from astrocytes, NOGO from myelin) Fibrosis	Cavity and Scar formation at the injury site (C) Fill cavity with cell transplants to bridge the lesion and improve cellular terrain Removal of scar tissue - Surgical removal or chemical breakdown Pharmacological intervention to reduce or counteract growth inhibitory molecules
Wallerian degeneration (D) Breakdown of axon Breakdown of myelin with release of myelin associated inhibitory factors (e.g. NOGO)	Wallerian degeneration (D) Pharmacological intervention with neuroprotective drugs to limit the extent of secondary degeneration
Postsynaptic target cell (E) Loss of innervation and trophic support from injured cell Subsequent anterograde transneuronal degeneration	Postsynaptic target cell (E) Neurotrophic drugs Enhance plasticity from uninjured cells to retain trophic support from alternative pathway Cell replacement strategies

Fig. 24. This schematic and accompanying table provides a frame of reference for discussion of current progress in the development of novel interventions for promoting repair in the nervous system with particular emphasis on the CNS. Various cellular responses to axotomy are illustrated and further listed in the left column. Some corresponding interventions are listed in the right column and are discussed in the text.

genetically engineered cell lines that are subsequently grafted into the CNS. Therapeutic genes also have been introduced directly into CNS neurons *in situ* by way of relatively benign viral platforms (i.e., vectors).

Using this gene delivery approach, cells can be programmed to contribute to their own rescue in an autocrine fashion or to the survival of surrounding neurons by way of a paracrine mechanism.

8.2. Molecular Enhancement of Neuronal Regenerative Mechanisms

The molecular foundation of neuronal growth is becoming rapidly advanced with the advent of new technologies that can define the complex underlying changes in gene expression and patterns of protein synthesis. The knowledge gained from these techniques will elucidate appropriate molecules to target for intervention. Some therapies may thus influence the synthesis and transport of cytoskeletal elements involved in maintaining axonal structure or for promoting growth cone mobility, as discussed earlier. One molecule of particular interest has been a growth-associated protein (GAP-43), which is produced by neurons during development and by mature neurons that are capable of sustaining renewed growth after axotomy. GAP-43 is conveyed from the cell body to the axon by fast axoplasmic transport and represents one of the more abundant constituents of growth cones. More molecules will be undoubtedly identified that play important roles in initiating and maintaining nerve regeneration. Neurotrophic factors may again play vital roles in gene delivery, as some investigations already suggest, because of their ability to trigger regeneration-friendly metabolic responses through intracellular signaling pathways. Whereas enhancing regenerative axonal growth in the CNS is a major therapeutic goal, it should be recognized that such growth, if not regulated, could result in the formation of inappropriate synaptic connections leading to ineffective or detrimental circuitry and maladaptive plasticity.

8.3. CNS Lesions can be Bridged with Cells Alone or in Combination with Neurotrophic Factors Provided Locally

As PNS-to-CNS grafting experiments discussed earlier have illustrated, neurons in the CNS have the potential to regenerate axons for long distances when presented with an appropriate tissue substratum. They also appear capable of making new connections with appropriate targets when given the opportunity. It follows that another focus of therapeutic development is to improve the cellular microenvironment at the lesion site. This involves providing a compatible substratum with appropriate neurotrophic factors, extracellular matrix proteins, and cell adhesion molecules. Several neural and non-neural cell types have been explored for their ability to serve as cellular bridges either alone or after genetic modification (see previous discussion), pharmacologic manipulation, or in combination with biocompatible scaffolding materials. Some

cells, such as Schwann cells, are independently capable of providing a cocktail of beneficial molecular attributes.

It is important to consider that the success of any bridging method will be dependent on the elements used and the inhibitory nature of the scar matrix at the borders of the lesion, as well as the nonpermissive nature of the CNS at more distant levels. For that reason, whereas Schwann cells are ideal for nurturing axonal elongation through a lesion, their disadvantage is they are recognized by astrocytes as non-CNS elements at host-graft interfaces across which axons within PNS bridges must extend and reenter the CNS. Consequently, Schwann cells do not become intimately integrated with the CNS parenchyma *per se*, and nonpermissive astroglial interactions or interfaces can thus arise (see Section 6.1) much like at the PNS-CNS transition zone described earlier.

Modulation of the nonpermissive extracellular matrix of glial scars has been reported to enhance axonal reentry into the CNS. Other studies have attempted to circumvent the Schwann cell–astroglial scar issue by using olfactory ensheathing cells (OECs), which are cells that envelope axons in the olfactory nerve. In many respects, OECs represent a Schwann cell–astrocyte hybrid that is favorably disposed to axonal growth as the olfactory nerve is one place in the nervous system where spontaneous regeneration is ongoing throughout life (see later). Furthermore, these cells (like Schwann cells) have other important properties including the capacity to form myelin around previously demyelinated CNS axons. An especially valuable advantage of OECs is they can be harvested from a patient who will later become the recipient of an OEC graft thereby negating the need for immunosuppression and its potential adverse effects. Some clinical trials worldwide have already been initiated with these cells in subjects with spinal cord injury (see Table 3); however, more basic science and clinical evidence is required.

8.4. Options Are Emerging for Improving the Cellular Milieu at and Distant from the Injury Site

Once growth cones traverse a lesion, they must navigate through an unfriendly CNS cellular environment composed of relatively intact CNS tissue as well as areas of Wallerian degeneration or more localized areas of neuronal death to reach their original or alternative postsynaptic sites. As noted earlier, there is increasing information about the identity and nature of nonpermissive molecules. In the case of myelin (see Section 6.2), strategies are being pursued to

immunologically neutralize those molecules, modify intracellular signaling mechanisms and associated effects on specific molecular pathways, or block myelin protein interactions with the Nogo-A receptor activity. Along similar lines, current efforts to circumvent the effects of glial scars on axonal outgrowth are directed at enzymatic digestion of inhibitory extracellular matrix molecules, such as CSPGs mentioned earlier (see Section 6.1). Among other considered strategies is the introduction of specific guidance molecules and other growth-promoting substances to enhance neuronal growth capacity, even under otherwise adverse conditions, and of specific guidance molecules to direct this growth to an appropriate target (Table 1).

9. CAN CELLS THAT DIE BECAUSE OF NECROSIS OR APOPTOSIS BE REPLACED?

9.1. *Neuronal Replacement with Fetal CNS Tissue Grafts*

Regardless of the efficacy of neuroprotection, it is inevitable that significant cell loss can be experienced due to necrosis alone. A long-held notion has been that we are essentially born with all the neurons we will have as they are unable to replace themselves. In that sense, CNS regeneration involving endogenous neurogenesis has been long considered highly unlikely. The only place in the mature nervous system where true regeneration occurs by the birth of new neurons is in the olfactory epithelium where olfactory receptor neurons turn over at the approximate rate of every 40 days.

However, it now appears that cell replacement is a more feasible objective in many neuropathologic conditions than was previously envisioned. One way this may be accomplished is through transplantation approaches involving cells from various sources, including embryonic stem cells or fetal and adult-derived neural or non-neural stem or stem-like cells. A variety of cell types, both normal and genetically modified, have been used to treat a range of experimental models of CNS disorders. Table 3 summarizes some of cells that have been investigated and indicates how some have been used clinically. It should be pointed out that by genetic modification, an implanted cell can be manipulated to produce growth-promoting molecules, further enhancing the potential for repair.

Many years of extensive research involving experimental transplants of fetal CNS tissue in animals and humans has provided an important template for neuronal replacement at both the basic science and clinical levels. A variety of experiments have shown, for example, that grafts of embryonic CNS tissue can mature into highly differentiated tissues (Fig. 25A) even having topographic features characteristic of their adult, *in situ* counterparts. Neuroanatomic methods also have demonstrated that axonal projections can develop between host and donor tissues with remarkable accuracy in some cases that could be the basis for the development of functional neural circuitries (Fig. 25B). Likewise, many examples have demonstrated graft-mediated amelioration of behavioral deficits in several experimental models of neurologic disease and trauma.

Table 3
Examples of Donor Tissue and Cellular Replacement Approaches for CNS Disease and Trauma

<i>Transplant (donor) cell types</i>	<i>Used in clinical trial to treat</i>
Peripheral nerve tissue	Spinal cord injury
Isolated Schwann cells	Multiple sclerosis
Fibroblasts	
Activated macrophages	Spinal cord injury
Olfactory ensheathing cells	Spinal cord injury
Fetal CNS allografts (human-to-human)	Parkinson's disease Huntington's disease Spinal cord injury
Fetal CNS xenografts (nonhuman-to-human)	
Embryonal teratocarcinoma-derived stem-like cells	Stroke
Adult neural precursors	
Bone marrow stromal cells (a.k.a. mesenchymal stem cells)	Stroke Spinal cord injury
Stem cells	
Genetically modified cells (including fibroblasts, Schwann cells, neural precursors and astrocytes)	

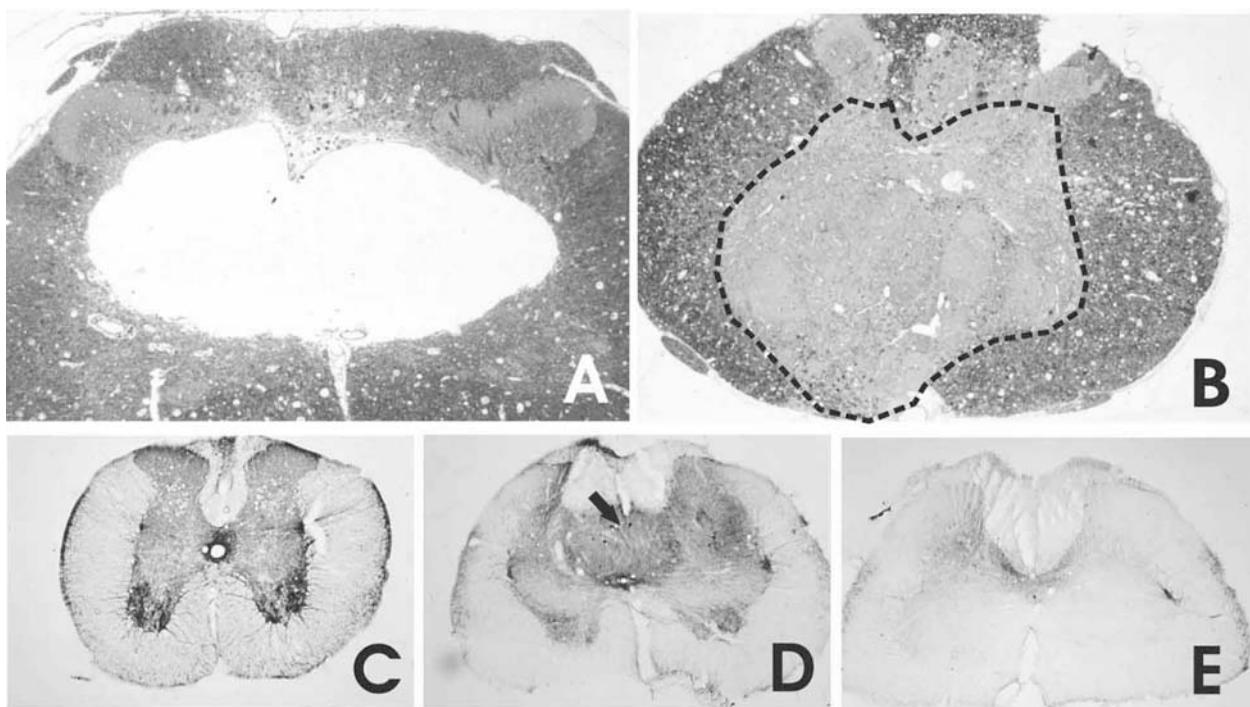


Fig. 25. As an example of cellular replacement potential in the CNS, two experimental results are presented in which fetal rat spinal cord tissue was grafted into two experimental models of adult rat spinal cord injury: a clinically relevant contusion (**A**) and a complete transection (**B**). (**C–E**) Can grafted neurons recognize appropriate targets? The focus of these images is on descending serotonergic (5-HT) innervation of the spinal cord. Using a 5-HT antibody, these bulbospinal projections can be visualized as darkly stained fibers. (**C**) 5-HT innervation of spinal gray matter normally entails projections to the intermediolateral cell column, dorsal horn, Rexed lamina X around the central canal, and ventral horn. After complete transection of the spinal cord, these projections are lost. (**D**) Transplantation of embryonic 5-HT neuron precursors in the dorsal column region caudal to the site of transection appears to reestablish considerably the normal 5-HT staining pattern. (**E**) This is even evident at a distance from the graft site, where in this case early projections to the intermediolateral cell column and dorsal horn are seen. Such findings suggest that some of the developmental targeting cues may be retained in the adult CNS and recognized by target-matched embryonic neuronal precursors. As a template for future applications of stem cells, results such as these are encouraging.

The potential for neuronal replacement via developing CNS tissue has been most extensively explored in relation to Parkinson's disease (PD). Here the rationale is to restore depleted levels of striatal dopamine (DA) due to progressive dopaminergic neuronal loss in the substantia nigra. Experiments in rodent models of PD showed, in fact, that embryonic DA neurons were capable of reversing motor deficits resulting from neurotoxic lesions of the host substantia nigra. Subsequent studies extended this approach to subhuman primates in which nigral dopaminergic neuronal loss and Parkinsonian symptoms were induced by the drug MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine), which is a byproduct associated with the illicit manufacture of a synthetic

opioid compound having effects similar to those caused by morphine or heroine. Preclinical data demonstrated that grafted fetal nigral cells can differentiate into *bona fide* DA-producing neurons, survive for long times, and most importantly contribute to improved locomotor function. These encouraging results ultimately led to clinical trials of human fetal DA cell transplantation in human PD subjects. Whereas some reports describe subjects who have shown remarkably positive outcomes, others report no statistical evidence for improvement.

Other clinical trials with fetal grafts also have been conducted in relation to Huntington's disease (HD) with long-term donor tissue survival having been reported. In one study, it was noted that host-graft

integration was very poor and could thus account for the failure to see any therapeutic effect. In contrast, another clinical trial indicated that neuronal replacement with fetal striatum led to cognitive improvements and neurologic stability for several years after transplantation before progressive deterioration reappeared.

Many logistical and biological challenges related to optimal transplantation conditions exist that are beyond the scope of this discussion. It is therefore not overly surprising that more consistent clinical benefits have not been seen in human subjects thus far. Nevertheless, evidence for graft survival and marked improvements in some human subjects has provided important proof-of-principle demonstrations for the feasibility of neuronal replacement in the injured or diseased CNS. Such findings have created considerable impetus for a currently burgeoning interest in the potential therapeutic promise of stem cell biology.

9.2. Stem Cells and Neuronal Replacement

As fetal CNS tissue is not an optimal therapeutic choice for various ethical and logistical reasons, more recent scientific efforts have turned to stem cells as sources of donor tissue for neural repair. One practical advantage of stem cells is that they represent self-renewing populations that can be expanded under tissue culture conditions and have the potential to give rise to a variety of neuronal phenotypes. The most robust type of stem cell is the *embryonic stem cell* (i.e., ES cell), which derives from the inner cell mass of the blastocyst. ES cells are pluripotent and thus capable of giving rise to cells in every organ system of the body. For neuronal replacement, a strategy would be to bank ES cells and harvest them as required. The cells would then be expanded and subsequently promoted to differentiate into specific neuronal phenotypes under defined molecular conditions in tissue culture. Those cells would then be grafted into the recipient brain where ideally they could further develop and ultimately become integrated with appropriate synaptic circuits. In doing so, they could promote behavioral improvements either alone or in conjunction with other interventions, including physical rehabilitation, exercise, or training in specific tasks. Promising experimental results have been obtained with this approach, and future clinical trials are envisioned. However, a large number of variables still need to be investigated to ensure safety and optimal use of ES cells.

The degree of “stemness” decreases as a function of developmental progression, such that the capacity for self-renewal and the differentiation potential of cells become increasingly more limited. Cells lose their pluripotentiality and become *multipotential* in that they can only give rise to cellular constituents of specific organ systems. Accordingly, only neuronal and glial *progenitors* derive from multipotent neuroepithelial cells of the developing brain and spinal cord. Though suitable for neuronal replacement, such human neural progenitors are more difficult to expand and perpetuate in tissue culture, and reliability of their differentiation into desired phenotypes also has proved challenging thus far.

A common disadvantage of human pluripotent ES cells and multipotent neural precursors that has led to considerable political and religious debate is their required derivation from embryos and fetuses. A provocative recent finding in that regard is that three scientific teams have independently demonstrated that pluripotency could be induced in adult skin fibroblasts by introducing a combination of specific transcription factors associated with ES cell pluripotency. Although clinical applications are still distant and require many technical issues to be resolved, the potential for reprogramming adult cells such that they can express a wide developmental repertoire offers an exciting alternative to current strategies optimally dependent upon ES cells.

The possibility also exists that cellular replacement could someday be facilitated through endogenous mechanisms involving resident populations of neural stem or progenitor cells. As noted before, endogenous neurogenesis in the adult mammalian nervous system has been largely limited to olfactory receptor neurons in the nasal epithelium. In non-mammalian species, however, neurogenesis has been seen in various regions of the nervous system. In goldfish, for example, new retinal ganglion cells and neurons in the optic tectum are born in large numbers throughout life. Certain songbirds also show significant neuronal replacement over the course of their lifetimes, which in some cases coincides with mating season cycles.

It is now recognized that stem cell populations also exist in the mature mammalian CNS, including the human brain, which have the capacity to give rise to new neurons both *in vivo* and in tissue culture. Thus far, these multipotent adult neural stem cells (aNSCs) have been only localized in two areas of the brain that represent specialized cellular and molecular milieus (i.e., *niches*) that can support self-renewal and differentiative potential. The first is a cellular region

subjacent to the ependymal lining of the lateral ventricles called the *subventricular zone* (SVZ), which has been illustrated in many original research publications, reviews, and textbooks. In brief, stem cells in the SVZ of the anterior lateral ventricles give rise to granule and periglomerular interneurons of the olfactory bulb. These newly generated neurons migrate from the SVZ to the olfactory bulb via a distinct pathway called the rostral migratory stream. The second aNSC niche is in the *subgranular zone* (SGZ) of the hippocampus (i.e., a region between the dentate gyrus and hilus). In both the SVZ and SGZ, new neurons appear to derive from a specialized population of astroglia in these stem cell niches. In contrast with other types of stem cells discussed, aNSCs are not as actively proliferative. Interestingly, these aNSCs exhibit many characteristics of adult astrocytes. At least certain astrocyte populations are thus precursors for newly born neurons in the adult mammalian brain.

Because these cells can be isolated from the mature brain and manipulated in tissue culture to form new neurons, one future possibility that has been considered is harvesting these cells from the person who would subsequently be the recipient. As discussed in relation to ES cells, the isolated aNSCs would be programmed to develop into a desired neuronal phenotype prior to transplantation. Such autologous grafts also would have the advantage of not requiring any form of immunosuppression.

Alternatively, one might imagine the possibility of having ways to selectively activate NSCs *in vivo* such that the brain would essentially repair itself. How to recruit such neuronal and glial progenitors for large-scale intrinsic tissue reconstruction is one of many challenges ahead. It is interesting, however, that correlations have been found between the level of new neuron production in the brain and certain environmental and behavioral variables. In fact, physical activity has been linked to neurogenesis in the mature hippocampus. The occurrence of neurogenesis in the adult brain and its enhancement by activity complements other lines of evidence for activity-dependent induction of neurotrophic factors and increased synaptic efficacy. The positive impact of activity on neurogenesis further underscores the important role that exercise and interactive rehabilitation can play in optimizing recovery from CNS injury or disease.

While both ideas are highly futuristic and an enormous amount of biological information must first be acquired, tremendous technology now exists and more is likely to emerge that can be employed to unravel the sundry complexities of stem cell biology.

From that perspective, such visions are less of a matter of fantasy and more of a part of the question “when” will such theoretical approaches materialize into clinical practice. However, an important side-note to the therapeutic potential of aNSCs that should not be overlooked is that they may also give rise to deleterious events, as evidence exists showing that certain brain cancers may evolve from cells with stem cell-like properties.

9.3. Stem Cells and Oligodendrocyte Replacement

Neurons are not the only cells in the CNS at risk. For example, oligodendrocytes are lost in the demyelinating disease multiple sclerosis (MS), as well as in many cases of blunt trauma. Tissue culture studies of cells from the adult CNS of rodents initially demonstrated the presence of oligodendrocyte progenitors using specific antibody markers for such cells. Similar methods of identification now demonstrate that oligodendroglial progenitor cells are also present in white matter of the normal adult human brain and spinal cord, as well as in the white matter of patients with multiple sclerosis. During the course of this disease, these progenitors appear to play a role in remyelination during remissions, but the extent of such spontaneous repair is suboptimal in most cases. Furthermore, in the chronic disease state, these cells decline in numbers. Whether the same is true under chronic trauma conditions is not entirely clear. Nevertheless, as in the case of aNSCs, the ideal would be to define strategies for activating oligodendrocyte progenitors *in situ* that could lead to more extensive and maintained remyelination. In addition to its relevance to action potential conduction, remyelination is important for maintaining axonal integrity in that long-term demyelination can lead to axonal discontinuity and subsequent degeneration. The fact that these precursors also can be maintained and expanded in tissue culture opens other transplantation possibilities. In fact, significant evidence exists showing that donor oligodendroglial progenitors or mature oligodendrocytes themselves are capable of successfully remyelinating axons in animals that are genetically lacking of normal developmental myelination and under experimental conditions resembling multiple sclerosis.

It has been shown that ES cells can be programmed to differentiate into oligodendroglial progenitors and that other cell types can achieve similar results. For example, OECs can produce myelin, and other lines of experimental evidence suggest that bone marrow

stromal cells can form functional myelin after transplantation into areas of CNS demyelination. An immediate and achievable short-term goal for human application is to define areas of focal demyelination that would have optimal functional benefits, such as in the spinal cord of multiple sclerosis patients. Because oligodendroglial precursors do not migrate extensively after transplantation, the longer-range goal is to define strategies for more widespread dissemination of donor cells not requiring extensively invasive approaches.

10. ARE CNS CIRCUITS AND PATHWAYS RIGIDLY WIRED?

Reflecting on earlier discussion of functional regeneration in the PNS, where directed axonal growth and the accuracy of target reinnervation are major considerations, the many possibilities for CNS repair (e.g., neuronal replacement) raise the issue of regenerative specificity even more profoundly. One might argue that in the periphery, musculoskeletal biomechanics place a much greater demand on the precision of motor axon regeneration alone. However, it is not difficult to envision what adverse functional consequences could emerge, for example, as a result of neuronal replacement, enhanced axonal regeneration, or enhanced plasticity with inappropriate circuit reinnervation in the CNS. A frequently identified potential adverse outcome considered with many repair strategies is the potential for generating pain among a host of other undesirable side-effects.

As many preceding portions of this book and the opening point of this chapter have underscored, the mature nervous system represents a constellation of intricate and highly orchestrated neural networks and pathways. In disease and after trauma, the finely tuned anatomic and functional integrity of the brain and spinal cord are disrupted. For all intents and purposes, such perturbations alter the internal environment, cellular constituents, and circuitry to such a great extent that it could almost be considered as a “new nervous system.” Although neuroplasticity can lead to some degree of spontaneous functional recovery maladaptive changes can emerge as a product of either endogenous self-repair mechanisms or therapeutic interventions. Therefore, how precisely do axonal pathways and neural circuits have to be reconstructed in order to achieve useful—but not necessarily perfect—functional improvements?

Although no absolute answer to this question currently exists, some experimental findings clearly

suggest that new circuits can be developed by physical training via directed rehabilitation and activity-dependent neuroplasticity. Other lines of evidence indicate that in certain circumstances, neuroplastic reserve may be so great that undamaged pathways may functionally substitute for lesioned axonal systems thus establishing “bypass” pathways. An illustration of this is provided in Fig. 26, which derives from recent experimental

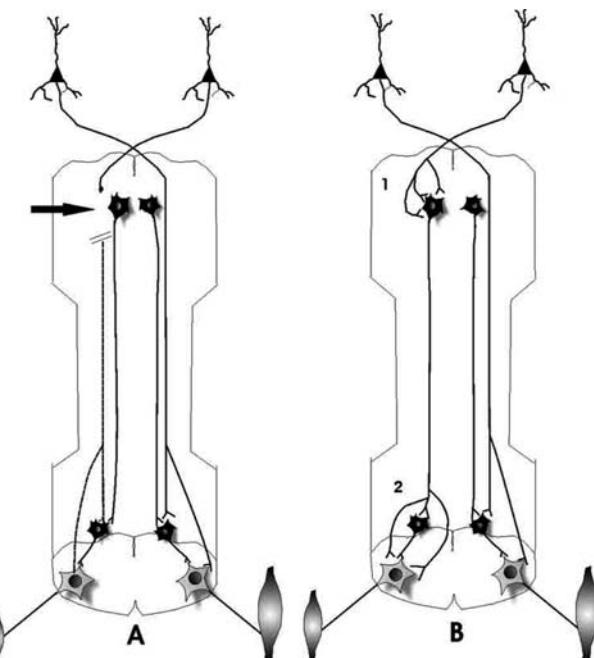


Fig. 26. An illustration of how alternative pathways may be recruited in the CNS to subserve functions lost as a matter of injury to primary axonal systems. This example is based on a study by Bareyre et al. (see Selected Readings) in which a partial spinal cord transection was made at a cervical spinal level that interrupted corticospinal tract (CST) projections to the lumbar spinal cord on both sides of the spinal cord but did not affect descending projections from long propriospinal neurons (**A**). For simplification, injury in this diagram is limited to one side. This lesion led to locomotor dysfunction as indicated by reduced muscle size relative to the uninjured side. (**B**) Although no evidence for regeneration of CST fibers was observed, neuroanatomic tracing results demonstrated sprouting of the injured CST axons, which then formed novel inputs to cervical propriospinal neurons (1). Likewise, the lumbar projections of these propriospinal cells exhibited sprouting in the lumbar spinal cord (2). Over time, extensive remodeling of this novel circuitry was shown; however, the “bypass” pathway provided by the long, descending propriospinal system illustrated resulted in significant recovery of ipsilateral function. Such findings are consistent with the fact that many spinal cord-injured individuals do show often subtle, yet observable progressive improvements in their neurologic status. These results also indicate that options for therapeutically promoting functional repair in the CNS may be substantially greater than former “hard-wiring” concepts suggested.

findings showing that after a partial spinal cord injury, which interrupts the corticospinal tract in the laboratory rat, hindlimb functional recovery is mediated by an alternative, undamaged descending pathway within the spinal cord that originates from interneurons above the lesion site. These long propriospinal neurons project onto circuits in the lumbar spinal cord that mediate hindlimb locomotion. The same circuits were targets of previously uninjured corticospinal axons. Recruitment of this novel pathway occurs through proximal sprouting of injured corticospinal axons above the lesion. The overall functional significance of this proximal fiber system response casts a different light on the concept of abortive regeneration. As an earlier, almost forgotten hypothesis once suggested, it may that with formation of novel synapses above the lesion neurons stop regenerating axons and return to gene expression patterns consistent with neurotransmission and maintenance of cellular integrity.

Such evidence for functional short-distance sprouting and detour pathway recruitment provides a provocative framework for appreciating how certain behavioral improvements may occur both in the spinal cord and brain in the absence of long-distance regeneration. In brief, findings such as this suggest that many previously unrecognized avenues for neural repair exist in the CNS. Unlike the case of peripheral nerve injury, alternative parallel pathways seem to be present that may make axonal regrowth and specificity of targeting less demanding than normal functional neuroanatomy suggests. Clearly, the CNS has the potential for adaptation via its propensity for circuit remodeling, and its collective efforts directed at self-repair may ultimately be complemented by other therapeutic strategies. Before such can be fully realized, however, more information needs to be obtained regarding where such plasticity is occurring in various functional domains, what the underlying mechanisms and circuitries are, and how to best target specific neuroplastic reserves.

11. SUMMARY

In this chapter, we have attempted to provide a comprehensive overview of basic principles related to neural tissue damage and repair. In so doing, details have been purposefully minimized in an effort to establish fundamental concepts and some accompanying clinical implications. For those wishing to pursue this topic further, the selected readings presented below offer more in-depth discussions of tissue dynamics and of cellular and molecular mechanisms.

An important goal was to identify major areas of emphasis in neuroscience research that were once clouded by pessimism and a sense of clinical surrender but that have now been challenged. Although functionally rebuilding an injured or diseased CNS remains a formidable objective, many lines of evidence, even if very conservatively interpreted, indicate that the “care but not cure” philosophy of past centuries is now being replaced by a paradigm shift in how scientists and clinicians view prospects for long-range improvements in neurologic outcomes.

Diverse neuronal responses to trauma have been identified. For those neurons that can survive injury, the capacity to regenerate effectively is dependent upon several intrinsic and extrinsic variables. In the PNS, achieving a high degree of functional improvement after peripheral nerve injuries is feasible, though not always achieved. Nevertheless, the PNS satisfies many of the basic prerequisites for regeneration. The issue of ultimate functional recovery is not purely a matter of the vigor and robustness of the regenerative response but is also a question of guidance and targeting. Intrinsically, PNS neurons and those CNS neurons that project axons to the periphery can exhibit considerable ability to initiate and maintain axonal regrowth over long distances. Unfortunately, this is not the case for CNS injury. However, it is encouraging that some neurons in the brain, spinal cord, and retina are capable of exhibiting robust regenerative responses when the cellular environment is rendered permissive.

The multidimensional nature of various neuronal and extraneuronal variables that dictate the success or failure of regeneration, and what has been learned about these factors, presents a variety of therapeutic targets. These options include strategies aimed at cellular, molecular, pharmacologic, and neurorehabilitative levels using a variety of sophisticated approaches. Such therapies are not mutually exclusive, and CNS repair will undoubtedly require interventions that use more than one modality, much like a designer therapy.

Viable options have begun to emerge with regard to neuronal death associated with injury or disease that can overcome another major obstacle to functional repair of the CNS. Whereas once neuronal replacement was considered highly unlikely, the reality of this approach now seems both rational and within reach. However, though there is a great deal of enthusiasm for using cellular repair strategies, there are many issues to be resolved. In addition, the notion of “self-repair” is sparked by advances in our appreciation for the neuroplastic reserve of the

CNS and techniques to encourage anatomically and functionally useful compensatory changes, including interactive training. As more is learned about the neuroplastic reserve of the CNS, greater chances will emerge for coupling promising interventions with spontaneous repair mechanisms.

In assessing future prospects, it is important to remember that the objective is not a matter of restoring normal functions, as much as it is an issue of achieving useful changes, no matter how large or small they may be. The recovery or preservation of even crude volitional movements or sensation can be meaningful to an individual's quality of life and in turn can signal important progress.

Although the challenges are formidable, progress made especially in the relatively short span of the past three decades or so has begun to shape dramatic new visions and approaches in the treatment of a broad spectrum of debilitating neurologic disorders. Such advances have captured the imagination of even the most conservative scientists and clinicians. Even more importantly, progress has raised the hopes of an increasingly more scientifically conversant patient population, as well as the interests of patient advocacy groups and members of the political sector. In that regard, an important aspect of modern-day medical practice and appropriate lay dissemination of scientific findings is to define ways to balance high expectations with the challenging biological realities of neural repair.

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Congenital Chromosomal and Genetic Abnormalities

Gregory Cooper and Gerald Eichhorn

Advances in genetics, dating back to the careful observations of Gregor Mendel, have shed much light on the etiology of inherited diseases. However, with this progress has come a realization that many of these conditions do not follow simple patterns of traditional Mendelian inheritance, and therefore a continual reshaping of our understanding of genetic disorders has taken place. Genetic disorders can be grouped into those linked to a single gene (monogenic) or multiple genes (oligogenic and polygenic) and to those related to larger chromosomal abnormalities. Genetic disorders can be further complicated by phenotypic variability despite an identical genotype. For example, multiple family members may carry an identical gene mutation but demonstrate varying clinical phenotypes. This suggests a role for additional modifier genes or other factors that might affect the clinical phenotype. Anticipation is another phenomenon sometimes seen in genetic diseases, such as Huntington's disease. In this illness, offspring of affected individuals might experience the onset of disease symptoms at a younger age. This appears to result from an increase in size of a triplet repeat segment within the mutant gene from one generation to the next.

DOWN SYNDROME RESULTS FROM DUPLICATION OF ALL OR PART OF CHROMOSOME 21

Chromosomal disorders result from changes in the total number of chromosomes or in structural rearrangements of the chromosomes. This can often be

evaluated by inspection of the karyotype. Structural abnormalities include deletions, insertions, translocations, inversions, and segmental duplications. Chromosomal disorders commonly manifest as mental retardation. One of the most common chromosomal disorders is Down syndrome, or trisomy 21. This condition is most commonly associated with an extra copy of chromosome 21, though in some cases, only a segment of the chromosome may be duplicated. The incidence of Down syndrome appears to be linked to maternal age at the time of conception, with a baseline risk of about 1:800 live births, increasing to about 1:110 at a maternal age of 40 years.

Individuals with Down syndrome have many typical facial features, including a rounded face and short nose with a flattened nasal bridge. The palate is typically high-arched. A short stature is usual, as is mental retardation with IQ scores ranging between 20 and 70. Interestingly, nearly all individuals with Down syndrome over the age of 30 years will demonstrate the typical neuropathologic findings of Alzheimer's disease (e.g., senile plaques and neurofibrillary tangles) at autopsy. This may be related to the presence of an extra copy of the gene encoding the amyloid precursor protein, which is normally found on chromosome 21. Mutations within this gene have been demonstrated in certain familial Alzheimer's disease pedigrees.

PRADER-WILLI AND ANGELMAN SYNDROMES RESULT FROM DELETIONS WITHIN CHROMOSOME 15

Both Prader-Willi and Angelman syndromes result from deletions within the same portion of chromosome 15 (15q11-13). The differential expression of these disparate syndromes is related to genomic imprinting. Prader-Willi syndrome initially manifests as neonatal hypotonia, feeding difficulties, and failure to thrive. Subsequently, at around the age of 1 to 2 years, developmental delay and childhood obesity are observed. By contrast, children with Angelman syndrome are said to show a resemblance to puppets, with flat heads, jerky movements, protruding tongues, and bouts of laughter. Developmental delay is also noted in this syndrome.

The phenotype caused by this chromosomal abnormality is related to the parent of origin, or genomic imprinting. If the deletion is inherited from the father, Prader-Willi syndrome is the result. If the deletion is inherited on the maternal copy of chromosome 15, Angelman syndrome is the result. Genomic imprinting is considered an epigenetic, non-Mendelian phenomenon. As a result of imprinting, genes may be inherited in either a silent or active state depending on the origin of that allele (e.g., maternal or paternal). Therefore, Prader-Willi syndrome is thought to result from the loss of paternally active genes, whereas Angelman syndrome results from the loss of a maternally active gene. It is thought that Prader-Willi syndrome results from a loss of expression of multiple contiguous genes, whereas Angelman syndrome may be related to loss of activity from a single gene locus.

THE SAME PHENOTYPE CAN BE CAUSED BY DIFFERENT MONOGENIC DISEASES

Depending upon the function of the gene involved, monogenic diseases can impair the function of multiple organ systems due to problems within a particular metabolic pathway. Alternatively, a particular disease phenotype can result from different monogenic diseases. This is illustrated in phenylketonuria (PKU). This condition, if untreated, can lead to multiple symptoms including mental retardation and seizures. PKU is caused by a defect in the enzyme phenylalanine hydroxylase in the great majority of cases. Fortunately, neonatal testing for this condition became available by 1960, and treatment through dietary restriction was found to prevent the

development of neurologic symptoms. However, about 1% of patients did not seem to respond to this treatment, leading to the realization that other genetic mutations might lead to the same disease phenotype. It was subsequently demonstrated that defects in either the phenylalanine hydroxylase pathway or in the tetrahydrobiopterin synthesis pathway could lead to phenylketonuria.

MULTIPLE GENES ARE INVOLVED IN OLIGOGENIC DISEASES

Becker and Duchenne muscular dystrophy are two genetic diseases that lead to progressive weakness in males. Both are X-linked and result from mutations of the DMD gene, with a resultant loss of functional dystrophin protein. Although both conditions lead to similar symptoms, Becker muscular dystrophy is generally considered the less severe of the two diseases, with a later onset of symptoms and an often less-rapid progression. However, it has been shown that a second genetic locus, the myogenic factor 6 (MYF6) locus, appears to act as a disease modifier in this condition, increasing severity. This locus is therefore considered a modifier gene. Modifier genes have been demonstrated in multiple other conditions, including cystic fibrosis, leading to the concept of oligogenic diseases. In fact, it has been suggested that genetic diseases exist on a spectrum from simple Mendelian diseases to oligogenic diseases to complex diseases involving many genes as well as environmental factors.

RETT SYNDROME IS A MONOGENIC DISEASE LEADING TO A COMPLEX DISEASE THROUGH AN EPIGENETIC PHENOMENON

Rett syndrome is a common cause of sporadic mental retardation with autistic features in girls. First described by Andreas Rett in 1966, it is classified as a pervasive developmental disorder. It is estimated that 1 in 10,000 to 15,000 girls develop Rett syndrome. Affected girls appear normal at birth and develop normally until 6 to 18 months of age. At that time, head circumference growth slows, ultimately leading to microcephaly. They regress in terms of language and cognitive abilities as well as motor and social skills. They typically develop stereotyped, wringing hand movements and autistic features. Seizures are common.

Rett syndrome is associated with mutations in an X-linked gene encoding the methyl-CpG-binding protein 2 (MeCP2) in the large majority of cases.

Missense, frameshift, and nonsense mutations as well as intragenic deletions have all been described. MeCP2 may affect gene expression in multiple ways. It binds to methylated CpG sequences of DNA and thereby functions as a transcriptional repressor. This may be through the recruitment and binding of a protein complex that alters chromatin structure. MeCP2 may also play a role in RNA splicing. The alteration of function of this protein in Rett syndrome likely leads to the misregulation of several

genes, thereby causing a complex disease through epigenetic mechanisms.

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Scott J. Russo and Colleen A. McClung

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1. INTRODUCTION

Drug addiction is a devastating disease that affects millions of people worldwide. Often, addiction to illicit drugs accompanies other types of mental illness such as depression, bipolar disorder, or schizophrenia, and the use of drugs can severely worsen these conditions. Drug use becomes classified as addiction when there is a loss of control of drug intake, resulting in negative consequences. These consequences can be health related but also include the loss of employment, family, and friends. In addition, individuals can become addicted to other activities such as gambling, shopping, or sexual activity, which can also lead to devastating consequences. Drug dependence is defined by the development of tolerance to the drug, as well as the presentation of physical withdrawal symptoms when drug use ceases. Addiction and dependence often go hand in hand, but an individual may be dependent on a drug (such as caffeine) and not necessarily show signs of addiction. In turn, drugs and other activities can be addictive without producing a physical dependence.

The risk of addiction is greater if drug use begins at an early age, if the individual is living in a stressful environment, or if there is a family history of addiction. Addiction is highly heritable, and studies in twins

find a much higher concordance among identical twins than among fraternal twins in the abuse of illicit drugs. From these studies, it is estimated that 60% to 80% of the differences in addiction rates between identical versus fraternal twins is due to genetic factors. This genetic influence is higher in males than in females and differs somewhat between specific types of drugs. The genes that lead to the vulnerability for addiction in humans are currently unknown, and the genetics is likely to be complex, perhaps involving many different genes. The most well documented gene mutations that influence addiction vulnerability are the variants of the aldehyde (ALDH) and alcohol (ADH) dehydrogenase loci found primarily in Asian populations. These variants lead to the “flushing syndrome” in which the individual’s skin becomes flush and they experience other unpleasant side effects after consuming alcohol. Because of these effects, these variants significantly reduce the risk for alcohol dependence and addiction.

Whole genome association studies are currently being undertaken in various human populations to identify other genes that may be responsible for an increased or decreased vulnerability for addiction. One recent study conducted by Uhl and colleagues (2006) examined 639,401 single nucleotide polymorphisms (SNPs) in individuals that displayed heavy lifetime use of illegal substances versus control individuals who had no history of drug abuse. They identified SNPs in roughly 50 genes that were significantly associated with drug addiction in both

European-American and African-American populations. These genes fall into many different functional categories, though many are associated with cell adhesion and communication. Similar whole genome scans are being conducted in mouse models of addiction to determine if there are overlaps between human studies and animal studies, which might help pinpoint the important genes in the addictive process.

1.1. The Importance of Dopamine

Brain imaging studies in human addicts have also provided useful information about the brain regions and genes that may be relevant in the development of addiction. These studies have found that all drugs of abuse lead to a large acute increase in dopamine transmission in limbic regions of the brain. This is seen in both addicted and nonaddicted subjects. However, individuals that have chronically abused drugs typically have a decrease in baseline dopamine neuronal activity in the midbrain and a decrease in dopamine D2 receptor availability in striatum. This effect is long-lasting and persists well after the subject has stopped taking drugs. Therefore, dopaminergic transmission is thought to be centrally involved in the addictive process.

The exact role of dopamine in addiction is still somewhat unclear. Though dopamine seems to be involved in the feelings of reward, or “liking” of certain stimuli, it is not necessarily needed for hedonic responses. The dopaminergic circuit centrally involved in drug reward between the ventral tegmental area (VTA) and nucleus accumbens (NAc) in rodents is diagrammed in Fig. 1. Mice that lack dopamine through various means still find sugar and other stimuli rewarding, and lesions of the VTA in rats do not abolish heroin self-administration. However, animals

that lack dopamine cannot use the feelings of reward to motivate goal-directed behavior. Therefore, dopamine may be more involved in reward-related learning. Natural reinforcers such as food, companionship, and sex, which are necessary for species survival, normally activate these dopaminergic circuits. It has been hypothesized that these circuits are involved in setting the reward value for stimuli based on the amount of reward that these stimuli have produced in the past. This then leads to the “wanting” and “seeking” of highly rewarding stimuli, and not necessarily the “liking” of the stimuli. In support of this theory, Schultz and colleagues (1993, 1997) recorded dopaminergic neurons in monkeys trained to expect the delivery of fruit juice after a cue. When juice is given on schedule after the cue, there is no increase in dopamine activity over baseline with the presentation of the juice. However, when the juice is given unexpectedly, this leads to an increase in firing. As well, the cells will fire at the earliest predictor of the juice, and if no juice is given at the expected time, then there is a suppression of dopamine firing. Therefore, when things are expected, then there is no increased dopamine, even though the juice is presumably still rewarding. However, if the reward is unexpected or if the reward does not follow the cue, then there are changes in dopamine signaling to reflect this new knowledge about the reward. Unfortunately, the dopamine signaling produced by drugs of abuse is many times higher than that produced by these natural rewards. Therefore, drugs of abuse produce a large response in the brain signaling that this is a substance of high reward value, even if the feelings of reward are no longer great. This alone will place the reward value of drugs above all other natural reinforcers. Furthermore, the long-term decrease in baseline dopaminergic activity in these circuits in drug addicts when the drug is not available also contributes to the loss of interest in activities that were once enjoyable, and an increased craving for drugs over everything else. In essence, the drugs have “hijacked” this reward circuit such that nothing else has value except for the drugs.

1.2. Other Important Circuits

Other circuits in the brain also contribute to the addicted state. Decreased activity in the orbitofrontal cortex (OFC) and cingulate gyrus (CG) have been consistently described in drug-addicted subjects. Interestingly, addicted subjects have an increase in activity in the OFC when presented with drug-associated stimuli, memories, or the drug itself. This enhanced activity is coupled with an increased desire for the drug. It is thought that this decreased activity during drug

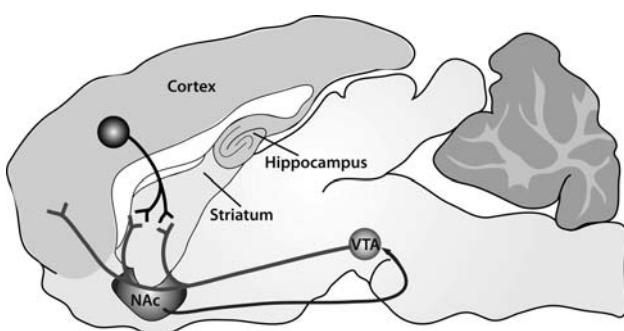


Fig. 1. Diagram of the rodent brain highlighting the connections between the VTA, NAc, and other regions. Glutamatergic connections from the cortex are indicated. The arrow represents the retrograde connection between the NAc and VTA.

abstinence and increased activity with drug and associated stimuli in these regions contributes to the enhanced motivation and compulsion to take the drug. Thus drug seeking occurs even when the drug is no longer perceived as pleasurable and its use could lead to adverse consequences. Indeed, hypermetabolism of these regions is associated with increased compulsive behavior in individuals with obsessive-compulsive disorders. In addition to the OFC, the prefrontal cortex, which is involved in higher reasoning, seems to also be disrupted in addicted individuals, and this likely contributes to the loss of inhibitory control over behavior. Moreover, regions of the brain known to be important for learning and memory such as the hippocampus and amygdala are more active in subjects with drug administration and cue-elicited drug craving, suggesting that the drugs and associated stimuli are activating long-term memories that may contribute to addiction.

1.3. Imaging Addiction Vulnerability

It is very difficult to determine the differences in individuals that lead to a vulnerability for drug addiction from brain imaging studies as differences between addicts and controls could come from the drug use itself and may not represent a preexisting condition. However, studies in nonaddicted subjects have found that baseline measures of striatal dopamine D2 receptors predict the response to the stimulant drug methylphenidate (MP). Those that described the drug as “pleasant” had significantly lower levels of D2 receptors than those that described the drug as “unpleasant.” Presumably, dopaminergic signaling only contributes to a pleasurable response up until a certain point, and with too much activity the response becomes aversive. Thus, the individuals with higher D2 levels quickly reach the aversive point with MP treatment whereas those with lower levels do not. This is supported in animal models of addiction where rats trained to self-administer alcohol will significantly reduce their intake when D2 receptors are upregulated in the NAc. It is possible that abnormally low levels of striatal D2 receptors will make individuals more susceptible to addiction whereas high levels of D2 receptors are protective.

2. TECHNIQUES USED TO STUDY ADDICTION

Animal models of addiction are very important in developing our understanding of the vulnerability for addiction, the changes in the brain that occur in

response to addictive drugs, and the potential success of therapeutic treatments for this disease. Models of associative and instrumental learning have been used to quantify the appetitive nature of various stimuli. The associative model, proposed to measure the rewarding properties of the stimulus, uses repeated pairings of a conditioned stimulus with an unconditioned stimulus to induce a conditioned response in the absence of the unconditioned stimulus. Alternatively, using the instrumental learning paradigm, a measure of reinforcement, an animal is trained and then required to perform a specific motor response in order to achieve the desired outcome. Under these circumstances, the frequency of responding increases as the strength of the rewarding stimulus increases and the behavior becomes more difficult to abstain from.

The brain’s reward circuit evolved to process information about natural rewards such as sex and food and to maintain behaviors that would influence fitness and ensure survival of the species. It is well established that sex, food, and exercise can produce goal-directed behaviors in both humans and animals. Studies have shown that male rodents will develop a conditioned place preference for an environment previously paired with natural rewards, such as a receptive female or food. Rodents will also press a lever for access to these same natural rewards. Intracranial self-stimulation (ICSS), which operates on the same basic principles of instrumental learning theory, has been used extensively to measure reinforcement-related brain circuitry in laboratory animals. With this paradigm, an animal can be trained to press a lever to deliver a current through an electrode implanted in various brain structures. Though first demonstrated by Olds and Milner in the septal area, animals will press a lever and self-stimulate in the VTA, NAc, prefrontal cortex (PFC), and hypothalamus.

During the 1960s and 1970s, models of associative and instrumental learning were rapidly evolved to measure the addictive potential of many drugs of abuse. Conditioned place preference (CPP), which is an associative model used to assess drug reward, is performed by establishing associations between environmental stimuli and a drug’s rewarding properties. The animal is then tested, in a drug-free state, for their preference of the environment previously associated with either drug or saline administration. The ease and sensitivity of this paradigm makes it a perfect candidate for larger screens of addictive substances. This technique also enables one to determine positive and aversive properties of drugs while allowing experimental control of drug associations.

More sophisticated models of addiction have also been developed that use the principles of instrumental learning. With these procedures, an animal is surgically implanted with a catheter directly into the jugular vein. An infusion pump containing either drug or saline is connected to the catheter, which is activated when the animal presses a lever located within an instrumental or operant learning box. These drug self-administration models are more relevant to addiction in humans and can be used to measure many phases of the addiction process including acquisition, maintenance, extinction, and relapse. In recent years, research has focused on understanding the molecular pathways that regulate these various phases of drug self-administration, with the hope of testing and developing medications to better treat addiction in humans.

Although models of CPP should be distinguished from self-administration, there is a large degree of overlap between compounds shown to induce both behaviors. These compounds include cocaine, morphine, amphetamine, alcohol, nicotine, cannabinoids, and 3,4-methylenedioxymethamphetamine (MDMA). Likewise, compounds such as dopamine, opioid and cholinergic receptor antagonists, as well as antidepressants, are not self-administered nor do they induce CPP. Despite these similarities, compounds such as lysergic acid diethylamine (LSD) and buspirone induce CPP but are not self-administered, and pentobarbital and phencyclidine are self-administered but do not induce CPP. In addition to these findings, recent studies have demonstrated a more convincing distinction between CPP and self-administration. One such study showed that individual differences in the magnitude of amphetamine CPP and rates of amphetamine self-administration were not correlated. Furthermore, the neuropharmacologic mechanisms that underlie CPP and self-administration do not always overlap. For example, dopamine D2 receptor antagonists attenuate cocaine self-administration but not cocaine CPP. Although certain dissociations exist between both methods of drug assessment, it is accepted that data from these studies mostly complement each other and add to our overall understanding of drug abuse.

Another well-known model to understand the long-term biological and behavioral changes that result from chronic drug intake is the behavioral sensitization model. With this paradigm, animals are given an injection of cocaine, and their activity is measured through the use of a photobeam or video-tracking activity monitor. On subsequent days, the

animal is again injected with the same dose of cocaine, and their activity will increase steadily across the chronic drug treatment. The increased locomotor activity is an incredibly stable form of behavioral plasticity that can last for many months. Given its stability, behavioral sensitization can be used to understand the changes that make addiction a life-long disease. For this purpose, sensitization models are used to determine biochemical/molecular changes as a function of this behavior plasticity. However, many argue the relevance of these behavioral changes, and caution should be taken regarding the direct implications about the addiction process. The sensitization paradigm is an attractive model for large-scale screens, and results from these studies largely overlap with place-conditioning and self-administration studies. Operating on similar brain circuitry, animals will sensitize to a wide range of addictive substances, and results from these specific drug studies will be discussed in detail below.

3. SEX DIFFERENCES AND THE NEUROENDOCRINE SYSTEM IN ADDICTION

There have been many recent reports in both humans and animals showing sex-related differences in certain aspects of the addiction process for nearly every drug of abuse. In humans, male subjects report greater intake of cocaine, nicotine, and alcohol with more episodes of euphoria or “good feeling” than do female subjects. Although there are no gender differences in drug-taking behaviors such as the time spent using, total amount used, and money spent, a study has shown that men remain abstinent longer than do women despite these reports of increased euphoria in men. Therefore, shorter abstinence periods in females may be related to an increased sensitivity to drug-conditioned stimuli and increased craving for the drug. Although females typically make up a lower percentage of total drug abusers, in controlled laboratory studies in rodents there is overwhelming evidence that females are more sensitive to the euphoric/rewarding effects of abused drugs and are more likely to transition to addiction when given access. This phenomenon has led many researchers to examine whether there is a hormonal basis for these differences. The most obvious hormonal difference between females and males is in levels of the gonadal steroids (estrogen, progesterone, and testosterone), all of which have been shown to affect some aspect of neuronal function in brain reward regions.

3.1. Neuroendocrine Regulation of Drug Sensitivity

The mechanisms of increased drug sensitivity could occur directly through modulation of the hypothalamic-pituitary-gonadal (HPG) axis to then regulate behavioral and neurochemical responses to drugs. Research into these effects has revealed that cocaine dramatically regulates hormone release at all levels of the HPG axis. Cocaine increases circulating levels of gonadotropin-releasing hormone (GnRH) from the hypothalamus and luteinizing hormone (LH) from the pituitary. Downstream of these factors, drugs of abuse influence levels of circulating estrogen and progesterone. Regulation of these hormones by cocaine seems to depend on the stage of the estrous cycle during which the drug is administered. For example, in the follicular phase but not in the midluteal phase of the rhesus monkey, cocaine increases plasma levels of estradiol. Alternatively, in male rats, cocaine significantly decreases both the synthesis and secretion of testosterone.

Gonadal hormones have a profound effect on neuronal plasticity and may represent one of the mechanisms by which cocaine influences neuronal activity. Therefore, sex differences in the hormonal profile of males and females would impact the cascade of events after cocaine administration. These differences may, in turn, explain behavioral and neurochemical changes during the female cycle, as well as sex differences in these responses. Indeed, it has been shown that hormonal fluctuations during the different stages of the menstrual cycle in humans results in significant behavioral and neurochemical changes. Women in the follicular phase show a higher peak in plasma cocaine levels than during the luteal phase. In rats, the stage of the estrous cycle influences an animal's motivation to self-administer psychostimulants such as cocaine and amphetamine. Rats in estrus also experience a greater intensity of drug-induced stereotyped behavior and locomotor activity than during other stages of the cycle. In order to avoid complication by the hormonal fluctuations associated with the estrous cycle, gonadectomized rats provide a model system to investigate the influence of gonadal hormones on drug-induced behaviors.

Both castration and ovariectomy affect the behavioral response to drugs of abuse. Ovariectomy decreases cocaine-induced locomotor activity, reward, and acquisition of self-administration. After ovariectomy, progesterone replacement inhibits drug-reward and reinforcement, whereas estrogen can fully restore these responses. In male rats, testosterone seems to

play a more limited role in drug-related behaviors. Testosterone administration lowers stereotyped activity such as head swaying, exploratory behavior, uncoordinated body movements, and the locomotor response to cocaine. However, surgical removal of the testes does not alter drug-induced CPP nor has it been shown to alter any phase of drug self-administration. Overall, these studies suggest that gonadal hormones in females play a pivotal role in the behavioral response to cocaine and again support our hypothesis that hormonal differences between males and females explain sex differences in drug-taking behaviors.

3.2. How Do Gonadal Hormones Influence Addiction?

Although studies into the molecular mechanisms of these sex differences have just begun, and therefore will not be discussed in detail here, a major focus of both current and past literature has been in the regulation of monoamines by gonadal hormones. Therefore, it has been proposed that sex and estrous-related differences in the dopamine system may be related to differences in hormonal regulatory mechanisms. Studies have shown that electrical stimulation of the mesolimbic dopamine system elicited sex- and estrous cycle-dependent differences in rotational behavior. Furthermore, females have greater release of dopamine in the striatum after an acute injection of amphetamine compared with males. In females, monoaminergic activity is altered by cyclical changes in gonadal hormones throughout the various stages of the estrous cycle, and ovariectomy results in a profound attenuation of monoaminergic neurotransmission in reward circuitry. Ovariectomy also decreases levels of dopamine receptors in the striatum and substantia nigra. Further evidence shows that estrogen replacement enhances dopamine receptor expression and dopamine transporter uptake sites in the striatum. Other monoamine systems show unique patterns of regulation by ovarian hormones. Specifically, ovariectomy-induced decreases in serotonin 2A receptor densities are restored to control levels after replacement with estrogen. Although not well characterized, progesterone has been shown to enhance the metabolism of both serotonin and dopamine and in conjunction with estrogen can alter total levels of dopamine in the NAc. Although the direct relationship between the HPG axis, monoamines, and drug reward is not known, there is some consensus that gonadal hormones modulate addictive behaviors through their actions on these monoamines within brain reward structures.

3.3. Influence of Other Hormones

Alternative hormonal mechanisms may also play a role in certain aspects of drug addiction in males and females. It is well established that females release greater levels of adrenocorticotropic hormone (ACTH) and corticosterone in response to environmental stressors compared with males. Interestingly, gonadal hormones are key factors in the reported sex differences in activity of the hypothalamic-pituitary-adrenal (HPA) axis. The corticotropin releasing factor (CRF) gene can be activated when estrogen binds to sequences of DNA known as estrogen response elements (EREs). Further evidence shows that increased HPA activity in response to stress in females can be abolished after ovariectomy. Interestingly, ovariectomy also decreases cocaine-induced release of corticosterone in females, whereas castration has no effect. Therefore, it has been proposed that interactions between the HPA and HPG axes in female rats may influence the behavioral response to cocaine and partly explain sex differences in these responses.

Environmental stressors that activate the HPA axis play a role in the onset of many psychological disorders dependent upon reward circuitry, including drug addiction, mood, and anxiety disorders. Activation of the HPA axis by cocaine leads to increased ACTH and corticosterone signaling. Although the HPA axis does not affect the rewarding properties of cocaine in either sex, as measured by CPP, induction of the HPA axis does affect the acquisition and reinstatement phase of drug self-administration. Furthermore, corticosterone replacement enhances behavioral sensitization to cocaine, whereas inhibition of corticosterone decreases both behavioral sensitization and reinstatement of cocaine self-administration. The implications of stress in the transition from drug use to addiction, along with sex-related differences in HPA axis activation, suggest that differences in these hormones may also underlie sex differences in certain aspects of the behavioral response to cocaine.

The role of adrenal hormones in regulating the monoamine system has also been shown. Adrenalectomy suppresses dopamine levels in the striatum and the shell of the NAc. Moreover, adrenalectomy has been shown to decrease the induction of Fos proteins in the striatum after D1 agonist binding. Both stress and glucocorticoid replacement induce dopamine release in the nucleus accumbens (NAc) and striatum. In turn, microinjections of a dopamine agonist and cocaine in the NAc, striatum, and medial prefrontal cortex increase plasma levels of corticosterone. Taken together, these results suggest that sex

differences in the regulation of monoamines by adrenal hormones may influence the behavioral response to psychostimulants.

4. CIRCADIAN RHYTHMS AND ADDICTION

Recent studies suggest that the circadian clock, which controls the sleep/wake cycle and other rhythms that cycle over 24 h, plays an important role in drug addiction. People who are addicted to drugs generally have major disruptions in their sleep/wake cycle, as well as abnormal rhythms in body temperature, hormone levels, and blood pressure. Some of these disruptions are caused by chronic exposure to the stimulant or depressant properties of the drugs themselves. Furthermore, withdrawal from these drugs usually leads to long-lasting effects on daily rhythms, and this often precipitates relapse. Addiction may also be more prevalent in individuals that have a compromised or nonfunctional circadian clock. People who have chronic sleep problems are more likely to become addicted to drugs. Drug use can also have a seasonal pattern with an increase in the use of alcohol and other drugs typically in the winter when certain individuals are more susceptible to depression. In addition, there is a diurnal variation in the sensitivity to nearly all types of illicit drugs in the human population. Retrospective studies that examine the admission of drug overdose victims to urban hospitals find the greatest presentation around 6:30 PM versus other times of day. This suggests that the response to drugs differs depending upon the time of day that the drug is taken. Indeed, studies in animal models of addiction have found a diurnal difference in drug sensitivity, sensitization, CPP and self-administration. Rats have an increase in the sensitivity to the reinforcing properties of cocaine at 1:00 AM and 1:00 PM versus rats tested at 7:00 AM or 7:00 PM as indicated by drug self-administration at lower doses and a decrease in the overall drug intake at these time periods. This difference in sensitivity is not due to changes in the pharmacokinetics of cocaine as they are similar at all times of day. Furthermore, behavioral sensitization in mice is greater when cocaine is given during the day than during the night, and this is dependent on the circadian hormone, melatonin. Intriguingly, other studies using a rat model have found that in contrast to short-term sensitization, long-term cocaine sensitization (observed 2 weeks after the last injection) is greater when the drug is given at the onset of the dark phase (ZT12). A single treatment with melatonin 15 min before the last cocaine injection at an earlier time

point (ZT6) enhanced this long-term sensitization 2 weeks later, suggesting that melatonin is involved in the long-term sensitization to cocaine. In addition to drug self-administration and locomotor sensitization, studies have found that CPP for cocaine also has a diurnal rhythm, and the effects are greater during the day (ZT5) versus the night (ZT20). This difference is also dependent on melatonin as pinealectomy abolishes this rhythm in response.

4.1. The Molecular Clocks

The molecular clocks in all organisms are essentially a series of interconnected transcriptional loops that are regulated over the course of 24 h. In mammals, the Circadian locomotor output cycles kaput (*CLOCK*) and Brain and muscle Arnt-like protein-1 (*BMAL1*) proteins dimerize and induce expression of the *Period* genes (*Per1*, *Per2*, and *Per3*), the *Cryptochromes* (*Cry1* and *Cry2*), and many other genes. After a time lag, the PER and CRY proteins enter the nucleus, and CRY proteins inhibit the actions of *CLOCK*:*BMAL1*. In forebrain regions or under conditions when *CLOCK* is nonfunctional, Neuronal PAS domain protein 2 (NPAS2), a protein very similar in sequence and function to *CLOCK*, can regulate the expression of the *Per* and *Cry* genes. NPAS2 has particularly high expression in striatal regions of the brain.

The master circadian pacemaker is located in the suprachiasmatic nucleus (SCN) at the base of the hypothalamus. However, these proteins are expressed throughout the brain, and they form SCN-independent pacemakers in other brain regions that control the entrainment to food and other non-photic stimuli. Indeed the circadian activity rhythms and molecular rhythms in striatal regions can be entrained to daytime injections of psychoactive drugs like methamphetamine, even in SCN lesioned (thus otherwise arrhythmic) animals. Treatment with methamphetamine shifts the expression of the *Per* genes in striatal regions, which presumably leads to the shift in activity rhythms, whereas molecular rhythms in the SCN and in melatonin levels remain unaffected by these treatments. Thus there is a disconnect between the SCN, molecular rhythms in the striatum, and locomotor activity rhythms with administration of addictive drugs. It is possible that the long-term desynchronization of the molecular clock in striatal regions from the SCN could lead to alterations in mood, motivation, or other processes associated with addiction.

4.2. Disruptions in Rhythms and Clock Genes Influence Drug Reward

Studies in animal models of addiction have found differences in free-running circadian rhythms between genetically inbred strains of rats and mice that display variations in drug preference. This suggests that the change in rhythms may contribute to the vulnerability for addiction. Rats that were selectively bred based on a high preference for ethanol versus a low preference for ethanol have a shorter free-running period when animals are housed in constant light. One of the lines (the HAD line) also display a “splitting” of circadian activity in that they show two distinct bouts of activity in constant light, which is not seen in the low ethanol preferring lines. A similar shortening of the free-running period was also found in ethanol-prefering mice compared with those selectively bred for low ethanol preference. These results suggest that genetic ethanol preference is associated with abnormal circadian rhythms.

The original studies suggesting that individual circadian genes may play a key role in drug responsiveness were performed in the fruit fly, *Drosophila*, and found that flies lacking a functional *Per*, *Clock*, *Cycle*, or *Doubletime* gene (but not the *Timeless* gene) all fail to sensitize to cocaine. It was later found that the disruption of individual circadian genes in mice leads to alterations in the reward value for cocaine, morphine, and alcohol. Interestingly, some mutations lead to an increase in preference for these drugs whereas others lead to a decrease in preference. These genes are also differentially regulated by psychoactive drugs in a variety of brain regions, indicating that they are perhaps involved in selective tasks in specific regions that may or may not be related to their regulation of circadian rhythms in the SCN. Human genetic studies are also beginning to find specific circadian genes that associate with addiction, and indeed a variant of the *Per2* gene significantly associates with heavy drinking in alcoholics.

4.3. Circadian Rhythms and Dopamine

It is unclear how circadian rhythms or the genes that make up the clock modulate the reward value of drugs of abuse, but it may involve their regulation of dopaminergic transmission. Virtually all elements of dopaminergic transmission have a diurnal rhythm including the expression of the dopamine receptors, the dopamine transporter (DAT), and the rate-limiting enzyme in dopamine synthesis, tyrosine hydroxylase (TH). Furthermore, rhythms in cocaine sensitivity correlate with diurnal alterations in postsynaptic levels of

dopamine and the activity of dopaminergic receptors in striatal regions. Some of the rhythms in gene expression in the VTA are regulated by the SCN whereas others are independent. All of the core clock components are expressed in the VTA and interestingly, mice that have a mutation in the *Clock* gene have an increase in dopamine cell firing and bursting in the VTA. This increase in dopaminergic activity correlates with their increased preference for cocaine and reward value for cocaine as measured by ICSS. Moreover, a number of gene expression changes in the VTA were noted in these mice including an upregulation of TH levels. In addition, restoration of a functional CLOCK protein specifically in the VTA of the *Clock* mutant mice rescues at least a portion of their abnormal behaviors. These results suggest that CLOCK functions in the VTA-NAc circuit to regulate dopaminergic synthesis, neuronal activity, and many of the behavioral responses associated with drug addiction. Additional studies will help determine the role of these individual circadian genes and the circadian clock as a whole in the development of addiction.

5. MECHANISMS OF ACTION FOR SPECIFIC ADDICTIVE DRUGS

5.1. Opiate and Opioid Drugs

Opiate and opioid drugs are derived from the opium poppy plant and include morphine, codeine, and heroin among others. The euphoric and analgesic properties of opium have been known for thousands of years, dating back to at least 4000 BCE. Originally, people drank the juice of the poppy to induce its effects. Later, the Chinese began to smoke opium preparations with or without tobacco, and opium became such a problem in China that two opium wars were fought in the mid-1800s in an effort to curb the opium trade between the British Empire and China. The active ingredient in opium, morphine, was discovered in the 1800s and later refined for medical use. Morphine is very effective in treating chronic pain and is still widely used today. Unfortunately, like opium, morphine can be highly addictive and is often abused by patients. Ironically, heroin, a derivative of morphine, was once marketed as a treatment for morphine addiction. However, it was soon realized that heroin has an even greater abuse potential than does morphine, and heroin continues to be one of the most widely abused and addictive drugs in the world.

All of the opiate and opioid drugs stimulate the G protein-coupled μ , κ , and δ opioid receptors in the

brain and spinal cord. Opioid receptors are coupled to the inhibitory G proteins, G_i/G_o . The endorphins and enkephalins are endogenous ligands for these receptors that exist in the brain. Endomorphin is thought to be the predominant ligand for the μ -opioid receptor (β -endorphin also binds this receptor), the enkephalins bind the δ -opioid receptors, and dynorphins bind κ -opioid receptors. In addition, there are a number of opioid peptides that are not selective for any one receptor. Physical dependence is very common with all of these drugs including the development of tolerance and a serious withdrawal state after drug intake has stopped, which includes chills, fever, nausea, vomiting, and diarrhea. Opioid antagonists such as naltrexone and naloxone block the action of morphine and other drugs at the opioid receptors, and treatment with these antagonists in morphine-dependent individuals or animals leads to the precipitation of a withdrawal state. Methadone, which is a much slower acting opioid drug with a half-life of 15 to 60 h, can be given to morphine or heroin addicts once daily to delay or prevent withdrawal. Methadone can also be effective at alleviating the intense cravings for heroin or other opioid drugs, and because of its pharmacology, it does not produce the quick "high" associated with these drugs that often leads to addiction.

Animals will readily self-administer these drugs and show preference for a drug-paired environment in CPP paradigms. The rewarding properties of opiate and opioid drugs are likely mediated, at least in part, through increased dopaminergic transmission between the VTA and NAc. A diagram illustrating the actions of these drugs and others on this circuit is shown in Fig. 2. Morphine and heroin administration in rats increases extracellular levels of dopamine in the NAc shell. This effect is partly mediated by activation of μ -opioid receptors on GABA interneurons, which leads to increases in VTA dopaminergic transmission. Endogenous opioid peptides in the VTA and NAc can also modulate dopaminergic transmission. Peptides derived from prodynorphin inhibit dopamine neurons, whereas increases in proenkephalin-derived peptides indirectly enhance dopaminergic signaling.

Opiate and opioid drugs can also act on opioid receptors located on accumbal neurons. Opioid receptor stimulation in the accumbens is thought to modulate some of the rewarding effects of these drugs, as well as some of the negative symptoms associated with withdrawal. Indeed, local injection of D2 dopamine receptor antagonists in the NAc shell can produce opiate withdrawal symptoms in

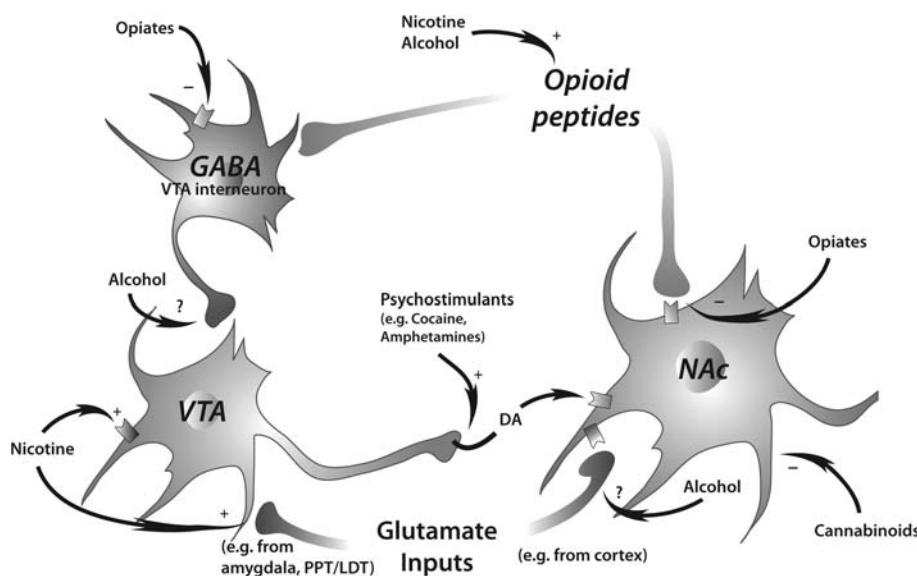


Fig. 2. Actions of several classes of addictive drugs in reward circuits. Opiates bind to the μ opiate receptors on GABAergic neurons in the VTA and NAc and inhibit their activity. This leads to greater activity of the dopaminergic neurons that are normally inhibited by GABA. Cocaine blocks the reuptake of dopamine into the presynaptic cell by the dopamine transporter, whereas amphetamines reverse the actions of the transporter. Both actions result in more dopamine at the synapse. Nicotine acts at the nAChRs on the dopaminergic neurons, leading to enhanced dopamine release. Alcohol acts at multiple sites in this circuit, most notably at GABA_A receptors. The cannabinoids (THC) bind CB1 receptors in the NAc and VTA leading to increased dopamine release. DA = dopamine, PPT/LDT = pedunculopontine and laterodorsal tegmental nuclei.

morphine-dependent animals, whereas activation of D2 receptors in this region prevents withdrawal.

The locus coeruleus (LC) is the major adrenergic center of the brain, and it has also been strongly implicated in the manifestation of morphine withdrawal. Both μ and κ opioid receptors are expressed in these neurons, and when stimulated by either opiates or endogenous peptides, there is an inhibition of adrenergic neuronal activity. One of the most well characterized responses in the LC after opiate or opioid treatment is the change in activity of the cAMP response element binding protein (CREB). CREB is a transcription factor that regulates the expression of many different genes. CREB is activated via phosphorylation of Ser-133 by protein kinase A (PKA) and other kinases. Activation of the μ -opioid receptor in the LC by acute morphine decreases adenylyl cyclase activity, reducing levels of cAMP, PKA activity, and ultimately phosphorylation of CREB. Chronic morphine treatment results in a tolerance to these effects, and CREB activity gradually returns to normal or even slightly elevated levels. However, precipitation of withdrawal by naloxone in morphine-dependent animals leads to a dramatic increase in PKA-dependent CREB phosphorylation. These changes in CREB activity lead to the altered gene expression responsible for certain

responses characteristic of physical dependence and withdrawal. Indeed, morphine-dependent mice that are lacking the α and δ isoforms of CREB have less severe withdrawal symptoms when treated with naloxone.

The endogenous cannabinoid system (which is involved in the response to marijuana) also appears to interact with the opioid system to regulate drug reward. Expression of endocannabinoids is altered in striatal, cortical, and hippocampal regions by morphine administration. In addition, Cannabinoid receptor 1 (CB1) knock-out mice will not self-administer morphine, and they have reduced withdrawal symptoms after morphine treatment has ceased. This effect seems selective for the opiate and opioid drugs as these mice readily self-administer cocaine, amphetamine, and nicotine to the same extent as wild-type mice. Administration of selective CB1 antagonists similarly reduces opiate reward-related behaviors, and interestingly, these antagonists have no effect on opiate-induced release of dopamine in the NAc, suggesting that the actions of CB1 on opiate-reward are independent of dopamine.

5.2. Psychostimulants

The most commonly abused psychostimulant drugs are cocaine, amphetamine, and methamphetamine.

Cocaine is derived from the coca plant, *Erythroxylon coca*. Indians in South America have chewed the leaves of this plant in order to produce a mild stimulant effect for thousands of years. The active component, cocaine, was isolated in the 1800s and was popularized by Sigmund Freud as a medical treatment for several types of ailments including asthma, depression, syphilis, and opiate addiction. Cocaine is also a very effective local anesthetic, due to its effects on sodium channels, and is still used for this purpose today. Cocaine can be snorted, injected, or the free-base form of cocaine known as “crack” can also be smoked.

Amphetamine was originally synthesized as a drug similar to ephedrine, which was used to treat asthma and other ailments. Amphetamines have been widely used as a weight-loss aid and to stimulate alertness. Amphetamine-like drugs such as methylphenidate are also used in the treatment of attention deficit/hyperactivity disorder. Methamphetamine can be synthesized from ephedrine or pseudoephedrine, ingredients often found in cold or allergy medications. Because it can be synthesized fairly easily, it has grown in popularity in the United States in recent years, particularly in rural areas.

Cocaine inhibits the reuptake of the monoamines dopamine, serotonin, and norepinephrine into the presynaptic terminal after release. This results in a prolonged action of these molecules at postsynaptic cells. Amphetamines can actually reverse the actions of these monoamine transporters such that additional monoamines are pumped into the synapse. Certain psychostimulants can also directly bind monoamine receptors and inhibit the action of monoamine oxidase, which normally breaks down these monoamines. Over time, these systems adapt to the higher levels of transmitter in the synapse, and this leads to dysphoria and other negative symptoms when individuals are not taking the drug.

Like the opiate and opioid drugs, animals will readily self-administer cocaine and amphetamine. In animal models, low doses of cocaine or amphetamines lead to a general increase in motor activity. Larger doses will lead to less locomotor activity and more stereotypic behaviors such as grooming, chewing, rearing, and head bobbing. Whereas continuous exposure to stimulant drugs leads to tolerance to their effects, small intermittent doses of these drugs can lead to the development of sensitization. Sensitization is observed when a low dose of cocaine or amphetamine is given once a day, and the same dose of drug leads to a greater behavioral response each day. This effect can

last for months after the last drug administration. It is thought that the development of sensitization is associated with the addictive process. In humans, stereotypic behaviors and cocaine-induced psychosis become more and more common after long-term, intermittent use. Sensitization can also describe the increases in drug wanting, craving, and seeking that occur over time in addicted individuals.

Most of the studies of psychostimulant action have focused on the dopaminergic pathway between the VTA and NAc. Interestingly, chronic exposure to psychostimulants (or nicotine) leads to long-lasting increases in dendritic arborization and the density of dendritic spines in the medium spiny neurons of the NAc. These morphologic changes are thought to be involved in the development of sensitization to stimulant drugs as well as addiction. Surprisingly, chronic exposure to opiate drugs actually leads to a decrease in these dendritic spines, and the importance of these responses to different types of drugs is not well understood.

The glutamatergic system is also known to be involved in the responses to cocaine and is particularly involved in drug-seeking behavior. This has been tested in reinstatement models in which animals are trained to self-administer cocaine, then the cocaine is removed and the behavior is extinguished over a period of days. Later, reinstatement of lever pressing commences in response to a cocaine infusion, stress, or a cue previously associated with cocaine. The PFC becomes active during drug reinstatement, and when the activity of the prefrontal cortex is disrupted, this blocks the reinstatement of cocaine-seeking behavior. The PFC sends glutamatergic projections to the NAc, and this connection in particular is involved in modulating relapse behavior. Interestingly, acute cocaine treatment does not influence glutamate release in the NAc in drug-naïve animals. However, it produces a large increase in glutamate release in animals that have experienced chronic cocaine, particularly when the cocaine is associated with environmental cues. Changes in glutamate receptors and in the Homer proteins that associate with glutamate receptors occur in response to chronic cocaine, and they facilitate the cocaine-induced changes in glutamatergic transmission.

There are currently no effective pharmacologic treatments for psychostimulant addiction. The most promising drug thus far in treating cocaine addiction may be disulfiram, which has been given to alcoholics for many years to produce a violent physical reaction in the body when the individual consumes alcohol. It

is still unclear as to why disulfiram may curb cocaine use, but it is thought that its actions on the dopamine- β -hydroxylase enzyme increase dopamine levels to a point where unpleasant side effects from cocaine become apparent, including high levels of anxiety and paranoia. Therefore the disulfiram makes the cocaine undesirable. Cognitive-behavioral approaches are currently used to help individuals recognize their addiction, avoid the triggers that lead to drug use, and provide coping skills to help overcome cravings. Efforts to curb psychostimulant addiction have also included the development of a cocaine vaccine. This vaccine leads to the production of drug-specific antibodies that bind to cocaine and prevent it from traveling to the brain. Thus it neutralizes the drug and prevents its actions. This vaccine does not remove the desire or craving for cocaine, and addicts may still take other drugs or very large amounts of cocaine. Therefore the success rate for the cocaine vaccine is inconclusive. All of the current treatments rely on the individual's desire to stop the addiction and comply with the treatment. Unfortunately, the rate of relapse in psychostimulant abusers is very high, and better treatments for the intense desire for these drugs are needed.

5.3. Nicotine

Tobacco use accounts for nearly half a million deaths in the United States each year with one in every six deaths attributed to cigarette smoking. In addition to cigarette smoking, tobacco can also be chewed or smoked from pipes and cigars, and all of these forms are known to cause cancer in either the lungs or mouth. The tobacco plant has been cultivated in the Americas since around 6000 BCE and was widely used and traded by the native people. The active addictive component of the tobacco plant is nicotine. Nicotine is classified as a stimulant drug as it increases heart rate, alertness, and blood pressure. At higher doses, nicotine can cause dizziness, nausea and vomiting, however, tolerance to these effects occurs quickly. Chronic nicotine exposure leads to physical dependence, and withdrawal symptoms include anxiety, irritability, headache, nausea, increased appetite, weight gain, and intense nicotine cravings.

Nicotine stimulates the nicotinic acetylcholine receptors (nAChRs) on both presynaptic and postsynaptic cells throughout the brain and other organs. The nAChRs are pentamers made up of various α and β subunits. There are at least three major subtypes of nAChRs in the brain: (1) the α -bungarotoxin-sensitive receptors, which contain $\alpha 7$ subunits, (2)

high-affinity receptors containing $\alpha 4$ and $\beta 2$ subunits, and (3) low-affinity receptors that contain $\alpha 3$ and possibly $\beta 4$ subunits. Tobacco-related concentrations of nicotine seem to preferentially impact the $\alpha 4\beta 2$ nAChR subtype with little effect on receptors that contain the $\alpha 3$ subunit. Imaging studies using ^3H -nicotine suggest that exposure to nicotine leads to a rapid activation and subsequent desensitization of nAChRs. However, over a period of hours, this is followed by an upregulation of active nAChRs on the cell surface. This receptor upregulation after nicotine administration is thought to be important in nicotine craving and withdrawal.

The dopamine cells in the VTA express $\alpha 4\beta 2$ receptors, and nicotine exposure leads to an increase in dopamine release in the NAc. This increase in dopamine is thought to underlie the rewarding effects of nicotine as blocking the actions of dopamine through receptor antagonists or lesions reduces self-administration of nicotine in animal models. Interestingly, nicotine can improve cognitive performance in both laboratory animals and in human patients with Alzheimer's disease, schizophrenia, and attention deficit/hyperactivity disorder. This effect is thought to involve the actions of nicotine on the ventral hippocampus as nicotinic antagonists given specifically in this region lead to significant impairments in working memory performance. The health risks that come from tobacco use and the addictive potential for nicotine far outweigh the benefits of this drug for working memory, however, researchers are searching for modified nAChR agonists that selectively influence memory.

The most commonly used treatment for nicotine addiction is nicotine replacement therapy. After the individual has stopped smoking, nicotine is provided from a skin patch, lozenge, or in chewing gum to lessen the withdrawal symptoms and cravings for cigarettes. The individual can then gradually lessen the amount of nicotine per day until they are free from withdrawal symptoms. Nicotine replacement therapy is the most effective when paired with behavioral therapy to break the routines or habits that are strongly associated with smoking. In addition, bupropion hydrochloride (also known as Zyban or Wellbutrin) can decrease nicotine withdrawal symptoms and reduce the cravings for nicotine. Bupropion is known to inhibit the actions of the dopamine and norepinephrine transporters, leading to increased action of these monoamines in the synapse. However, its binding is relatively weak when compared with cocaine, thus it does not produce the same euphoric

and addictive effects. Bupropion may also act as a partial antagonist at the nAChRs, thus blunting the actions of nicotine on these receptors. Its effects were originally discovered when smokers who were taking this drug for depression also noticed that they had lost the desire for cigarettes. It gained U.S. Food and Drug Administration (FDA) approval in 1997 as the first treatment for nicotine dependence that does not contain nicotine. The effects of bupropion on nicotine addiction appear to be rather selective, and it is not significantly effective in treating addictions to other substances. However, individual cases in which alcoholics and psychostimulant users benefit from bupropion have been reported, and these studies are still ongoing.

5.4. Sedatives/Hypnotics

The sedative/hypnotics, mainly used to treat anxiety and sleep disorders, include benzodiazepines such as diazepam and alprazolam (also known as Valium and Xanax), and barbiturates such as chloral hydrate. Barbiturates first became available and rapidly gained popularity as a sleep aid in the early 1900 s. Benzodiazepines, which were introduced during the late 1950 s, rapidly replaced the use of barbiturates, becoming the main pharmacologic treatments for anxiety disorders. Barbiturates were replaced because they tend to be more addictive and produce a dangerous withdrawal syndrome with a greater risk of death. It was originally thought that these substances were not addictive, however, more recent evidence indicates a large risk for dependence as the length of treatment and dose increases. Other factors contributing to the abuse potential of these drugs are their interactions with alcohol and other CNS depressants which act through similar neurochemical mechanisms. The combination of alcohol with barbiturates or benzodiazepines will intensify their effects and can increase addiction liability, overdose, and ultimately death. Because of the strong physical dependence that is produced by these substances, the first approach to treatment is a gradual detoxification monitored by a physician followed by behavioral therapy and drug counseling.

Benzodiazepines and barbiturates produce their sedative-like effects through similar neurochemical actions within the brain. The major neurochemical substrates of these agents are gamma-aminobutyric acid (GABA) receptors. GABA is the most abundant inhibitory neurotransmitter in the central nervous system. The GABA receptors are made up of two major subclasses; GABA_A, which is a ligand-gated chloride

channel, and GABA_B, which is a G protein-coupled receptor. Barbiturates and benzodiazepines target specific sites on the GABA_A receptor to enhance inhibitory tone within the brain. They do so indirectly by causing allosteric changes in the receptor itself to unmask the site for endogenous GABA to bind and activate the receptor. Benzodiazepines and barbiturates are contraindicated for use with a number of over-the-counter and prescription drugs that can potentiate the sedative effects of this class of drugs. However, when used appropriately, the indirect mechanism of action is less likely than direct GABA agonists to cause death by overdose.

5.5. Cannabinoids and Hallucinogenic Drugs

The hallucinogenic or “psychedelic” properties of psychoactive drugs have been used historically to expand the mind and create an altered state of consciousness. The particular hallucinogenic drugs discussed here include cannabinoids such as delta-9-tetrahydrocannabinol (THC), one of the active compounds found in marijuana, lysergic acid diethylamide (LSD), and 3,4-methylenedioxymethamphetamine (MDMA). The hallucinogenic effects of these drugs have been suggested to act through the serotonin system; however, the addictive potential of these substances is hypothesized to include dopaminergic reward circuitry.

The use of cannabis as a mind-altering agent is believed to date back 4000 years to central Asia. Jacques-Joseph Moreau, a French physician, was the first to meticulously record the acute effects of THC, which include hunger, behavioral disinhibition, cognitive deficits, impaired motor function, and altered sensory perception or hallucinations. Cannabis, though largely thought of as a recreational drug, has been used medicinally for thousands of years and currently is being used to treat emesis (nausea and vomiting) in cancer patients, as an appetite stimulant in AIDS patients, and in glaucoma. However, its limited yet successful use medicinally has been overshadowed by an ongoing debate as to whether THC is a prototypical drug of abuse. Historically, research into the rewarding and reinforcing properties of THC have produced contradictory results. However, more recent studies have shown that like other drugs of abuse, THC is rewarding and reinforcing and can produce dependence, tolerance, and withdrawal-like symptoms upon cessation of chronic use. The development of cross-tolerance to other drugs of abuse may represent a mechanism by which THC can serve as a “gateway” drug to other more addictive substances.

Although researchers are just beginning to understand the cellular mechanisms of THC action, the circuitry and target genes affected are similar to other prototypical drugs of abuse. It has been shown that THC binds throughout the brain to a novel receptor, the cannabinoid (CB1) receptor, which has recently been cloned and characterized. The CB1 receptor is a G protein-coupled receptor and regulates almost all of the behaviorally relevant actions of THC. Animal models of cannabinoid reward/reinforcement also indicate the necessity of this receptor in drug action. CB1 agonists can induce CPP much like THC and CB1 antagonists block THC-induced CPP. Likewise, a genetically altered mouse, lacking the CB1 receptor, shows decreased reward and reinforcement to most drugs of abuse. CB1 activation also stimulates appetite, and the CB1 antagonist, rimonabant, has been effective in clinical trials to treat obesity. However, use of a CB1 antagonist as a clinical treatment for any disorder has been inhibited because they produce unwanted side effects on mood and anxiety.

LSD, first synthesized by the Swiss chemist Albert Hoffman, is a drug that produces extremely potent and more intense perceptual distortions than most other mind-altering drugs. LSD intoxication referred to by users as “tripping” produces both visual and auditory hallucination, and in some cases the user associates this experience with a positive “good trip” or negative “bad trip.” Though LSD is characterized by rapid tolerance and cross-tolerance to other hallucinogens (not discussed here), it does not cause dependence or withdrawal upon cessation of use. Further evaluation into the addictive properties of LSD shows it to be rewarding but not reinforcing. Therefore, LSD is not considered an addictive substance in that it does not promote drug self-administration.

The Merck Company first synthesized MDMA by mistake in 1912. MDMA was then forgotten or at least undocumented until the 1950s. During this time, research on MDMA was commissioned by the U.S. Central Intelligence Agency and the U.S. Army’s Chemical Warfare division to be used on prisoners of war as a truth serum. During the 1980s, MDMA or its more commonly known street name, “ecstasy,” gained popularity among recreational users. The popularity of MDMA spread at drug parties known as “raves” where users could dance and enjoy perceptual distortions while “high” on the drug. The negative health consequences of MDMA were quickly realized, and the drug was made illegal by the government in 1985. A number of press reports

indicated increasing deaths among MDMA users, due in part to heat exhaustion and dehydration during raves. As an amphetamine derivative with stimulant-like psychoactive effects, MDMA intoxication is characterized by a combination of perceptual distortions and positive mood indicative of its actions within brain reward structures. Unlike LSD, MDMA has been shown in laboratory studies to be rewarding and reinforcing, and it has been hypothesized that the reinforcing effects of MDMA may be through the amphetamine-like stimulation of dopamine neurons.

5.6. Alcohol

Alcohol has been abused for tens of thousands of years with recorded use dating back to the late Stone Age. As evidenced by the discovery of beer jugs used to consume fermented beverages, it has been suggested that alcohol existed as early as the Neolithic period (ca. 10,000 BCE). Today, alcoholism accounts for nearly 100,000 deaths each year, and the treatment of alcohol-related diseases costs nearly \$22.5 billion annually, representing a tremendous burden to our health care resources. Many serious health problems are associated with chronic long-term use, including both physical (gastritis, cirrhosis, malnutrition) and neurologic symptoms (dementia and a wide range of mood and anxiety disorders). Behaviorally, alcoholism is characterized by uncontrollable drinking and chronic relapse after periods of sobriety. It has been hypothesized that this vicious cycle of heavy drinking followed by abstinence is key to the process of addiction. Understanding the biological mechanisms of alcoholism to develop novel treatments represents a major goal of current research. Unfortunately, animal models of alcoholism have historically presented a logistical problem for large-scale research. Much like humans, alcohol-naïve animals will not self-administer the drug, due in part to taste aversion. Therefore, traditional methods have used sweeteners to mask the taste while slowly increasing the alcohol concentration until the animals will self-administer the drug alone. These methods, though successful, are time consuming, and the high levels of ingested sugar can confound biochemical analysis of brain tissue. More recently, vaporizers have been developed to deliver alcohol into the air. Chronic exposure to alcohol through this method rapidly produces an alcohol-dependent state. After a brief withdrawal period, animals will voluntarily administer ethanol. With this novel animal model of alcohol dependence, researchers are now better equipped to understand

the process of alcohol addiction as well as the biological mechanisms through which it occurs.

Like the sedative/hypnotic class of drugs, alcohol exerts its main effects through actions at the GABA_A receptor to increase inhibitory tone within the brain. Specifically, alcohol binds to the receptor causing a conformational change in the receptor that allows for ions to flow across the membrane causing the neuron to become activated. A number of recent studies have shown that the progression to alcoholism can be blunted or completely absent in mice that have been genetically engineered to express an inactive mutant of this GABA_A receptor subunit. Not surprisingly, these mutations will also change the animal's response to benzodiazepines, which act upon the same receptor. Although primary neuroanatomic structures mediating alcohol reinforcement are not fully understood, it is thought that alcohol may affect dopamine signaling in the VTA-NAc circuit indirectly. Evidence for this comes from studies in which infusions of the opioid receptor antagonist naltrexone, which acts mainly on VTA-NAc circuitry, can block ethanol reinforcement in animals. These findings resulted in a recent approval by the FDA to use naltrexone as a treatment for alcoholism in humans.

Corticosterone releasing factor (CRF) is a peptide originally described as a hypothalamic release factor to stimulate ACTH and corticosterone in response to stress. Peptide mapping studies have revealed high levels of CRF in the paraventricular nucleus of the hypothalamus (PVN), central nucleus of the amygdala (CeA), and bed nucleus of the stria terminalis (BNST), all of which are key limbic regions well-known for their role in addiction and processing of emotional information. CRF has been shown to play a role in the anxiety phenotype induced by acute withdrawal from alcohol as well as dependence and relapse. Studies have shown that peptide antagonists of CRF can block excessive drinking in the postdependent rat and also block stress-induced relapse. Given the high levels of CRF in the amygdala and its known role in stress-induced relapse, researchers have examined levels of CRF peptide in the brain of alcohol-dependent rats. They found that during acute withdrawal from alcohol, CRF levels in tissue were reduced. This effect then rebounds and CRF levels increase above baseline after 6 weeks of withdrawal. These data suggest that elevated availability of CRF during long-term withdrawal may be a key factor driving the increased sensitivity to stress-induced relapse and is an attractive candidate for drug development.

In addition to naltrexone, disulfiram (marketed as Antabuse) can be given to alcoholics to stop their ability to drink. Disulfiram blocks the oxidation of alcohol at the acetaldehyde stage. Accumulation of acetaldehyde in the blood leads to intense symptoms including flushing, throbbing headache, vomiting, respiratory difficulty, vertigo, tachycardia, and hyperventilation. Disulfiram is very slowly absorbed and eliminated from the body, therefore, it can produce unpleasant symptoms in response to alcohol for up to 2 weeks. In addition, the longer the patient stays on disulfiram, the more sensitive they become to alcohol. Disulfiram is only effective at preventing relapse if the individual complies with the treatment regimen, and it does not necessarily alleviate the cravings for alcohol. Other treatments for alcoholism include 12-step programs such as Alcoholics Anonymous and other behavioral therapies.

6. GENERAL MOLECULAR AND CELLULAR MECHANISMS OF ADDICTION

Drug addiction is thought to be the result of long-lasting changes in the brain that occur in response to chronic drug use that inappropriately strengthen certain circuits while diminishing the signal of others. The ability of the brain to adapt and change is known as neuronal plasticity. This plasticity is important for all types of learning and memory. Unfortunately, the chronic exposure to drugs also leads to the formation of "reward-related memories" that can make the drug nearly impossible to resist. The best-characterized potential cellular mechanisms of plasticity are long-term potentiation (LTP) and long-term depression (LTD). LTP and LTD describe long-lasting changes in synaptic transmission in response to repeated stimulation. Both LTP and LTD seem to be important in the development of all types of memory formation including the reward-related memories associated with drug addiction. With LTP there is an increase in synaptic strength over baseline with chronic stimulation. Changes in synaptic strength can be measured by assessing the synaptic currents mediated by alpha-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid (AMPA) glutamate receptors versus N-methyl-D-aspartate (NMDA) glutamate receptors in cells. Chronic cocaine, amphetamine, nicotine, morphine, or ethanol treatment leads to an increase in the AMPA/NMDA ratio and LTP in the dopaminergic cells of the VTA. This change lasts from 5 to 10 days after drug use ceases, indicating that these changes are lasting, but are not permanent. Nonaddictive drugs

such as fluoxetine and carbamazepine do not lead to LTP in dopaminergic neurons, suggesting that this change is important in the process of addiction. Indeed, animals that cannot elicit LTP in response to drugs of abuse have deficits in CPP for drugs and behavioral sensitization. In addition, animals that have an increase in the AMPA receptor, GluR1, specifically in the VTA, show enhanced responses to drugs of abuse, suggesting that they find the drugs more rewarding.

6.1. The Response to Drugs Is Regulated by CREB

Long-term neuronal plasticity requires new gene expression. This new expression is regulated by a series of transcription factors that become active in response to drugs of abuse. A diagram illustrating some of the changes that occur in the VTA-NAc circuit in response to drugs is shown in Fig 3. The most well studied transcription factor in drug

addiction is CREB. CREB binds to the cAMP response element (CRE) in many gene promoters, including several growth factors, enzymes, structural proteins, and other transcription factors. CREB activity is increased in limbic regions of the brain after acute or chronic treatment with opiate drugs or psychostimulants. This induction of CREB activity appears to be involved in regulating levels of drug reward. Activation of CREB in the VTA leads to an increase in GluR1 receptors, which likely contributes to drug-induced LTP in this region. However, the actions of CREB in the VTA are complex as CREB overexpression in the rostral versus caudal subregions of the VTA has opposing effects on opiate and psychostimulant reward. This may be due to differences in the proportion of dopaminergic and GABAergic neurons in these two subregions of the VTA. Several studies using inducible transgenic mice or viral-mediated gene transfer have found that

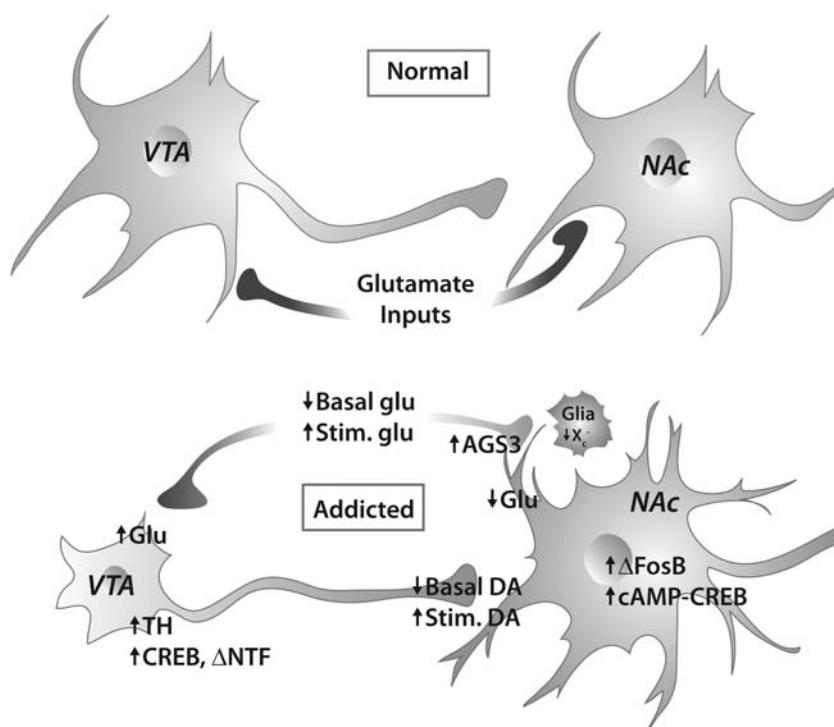


Fig. 3. A simplified comparison between the normal and addicted state of the VTA-NAc reward circuit, highlighting some of the important molecular and cellular changes that occur with chronic drug use. In the addicted state, changes in the levels of TH, CREB, neurotrophic factors (NTF), and other molecules are prominent in the VTA. In the NAc, changes in CREB and Δ FosB modulate the functions of these neurons. There is an overall decrease in the basal levels of both glutamatergic and dopaminergic transmission, which likely associate with withdrawal and craving, and there is an increase in the amount of stimulated glutamate and dopamine with drug use, which is associated with an inappropriately strengthened reward-related memory. The glutamatergic changes are mediated in part through upregulation of activator of G-protein signaling 3 (AGS3) in the cortex and a downregulation of the cystine-glutamate transporter system (X_c^-) in glia. Certain drugs of abuse also shrink the size of the VTA dopamine neurons (mainly opiate drugs), and others lead to an increase in dendritic outgrowth and spine number in NAc neurons (psychostimulants and nicotine).

increasing CREB levels in the NAc decreases the conditioned preference for drugs, whereas blocking CREB function through expression of a dominant-negative protein has the opposite effect. Furthermore, induction of CREB increases the excitability of GABAergic NAc neurons, whereas the dominant-negative CREB (mCREB) decreases neuronal excitability. It is thought that these acute actions of CREB in response to drug treatment results in a feedback mechanism that enhances GABAergic transmission leading to a decrease in dopaminergic activity. This produces drug tolerance and dependence, as manifested in dysphoria during withdrawal. As well, one of the target genes of CREB, *prodynorphin*, encodes a peptide (dynorphin) that is released from NAc neurons into the VTA where it binds to κ opioid receptors on dopaminergic neurons and decreases dopamine release. Therefore, CREB in the NAc is involved in reducing dopaminergic transmission and may represent an attempt at homeostasis after the large increase in dopamine occurring after treatment with drugs of abuse.

6.2. *ΔFosB* Regulates the Long-term Plasticity Associated with Addiction

The Fos family of transcription factors, including cFos, FosB, ΔFosB, Fos-related antigen 1 (Fra1), and Fra2, dimerize with Jun proteins to form an AP-1 transcription factor complex. Most Fos family proteins are induced rapidly in many brain regions after treatment with drugs of abuse. However, induction of cFos and FosB is very transient, and levels return to normal after a few hours. Furthermore, there is a tolerance to this induction with chronic treatments, and the induction becomes less and less prominent. In contrast, a splice variant of the *FosB* gene, ΔFosB, is slow to turn on, but it accumulates with chronic treatments due to its unusually long protein stability. Because this protein is only induced with chronic treatments and it is so persistent, it is thought that it is important in the plasticity associated with drug addiction. Long-term overexpression of ΔFosB specifically in the NAc of transgenic mice produces an overall addiction-like phenotype in response to several different drugs of abuse. This includes an increased preference for drugs and an increased motivation for drug self-administration. These mice also have an increase in their preference for natural rewards such as voluntary wheel running and sugar. Conversely, the expression of a dominant negative c-Jun (termed Δc-Jun) in the NAc opposes this addiction-like phenotype. ΔFosB is phosphorylated by casein kinase 2

(CK2) at serine 27, and this phosphorylation event contributes to ΔFosB's unusual stability. LTP induction rapidly increases the activity of CK2 in the hippocampus, thus it is possible that the activity of ΔFosB is increased and stabilized by CK2 after chronic stimulation, leading to some of the long-term gene expression changes responsible for plasticity. Thus ΔFosB represents an important regulator of gene expression involved in the long-term plasticity associated with addiction.

6.3. Modifications to DNA Contribute to Addiction

In addition to the functions of these specific transcription factors after chronic treatment with drugs of abuse, it is becoming clear that long-term modifications to the DNA also occur, and this results in changes in gene expression that are associated with addiction. DNA is tightly packed around octamers of the histone proteins H2A, H2B, H3, and H4, linked together by histone H1, forming the chromatin structure. Histone proteins can be modified to allow the DNA to unwind and permit transcription factor binding and gene activation, or the DNA becomes more tightly packed to inhibit transcription factor binding and prevent transcription. Gene activation is mediated through histone acetylation catalyzed by a histone acetyltransferase (HAT) enzyme. Histone deacetylase (HDAC) proteins reduce gene expression by deacetylating the histones. Histone phosphorylation is also generally associated with an increase in transcription, and histone methylation generally results in a decrease in transcription.

Chromatin remodeling through histone modification appears to play a role in drug addiction. One of the central features of addiction is that it is more likely to develop if drug use begins at an early age, indicating long-lasting changes in brain function. Studies have found that adolescent rats exposed to chronic cocaine have an increased response to cocaine in adulthood and an overall decrease in histone H3 methylation in the medial prefrontal cortex. This would lead to the persistent expression of genes in this region that are normally silenced. Treatment with drugs of abuse in adult animals also leads to histone acetylation at specific gene promoters, and the administration of an inhibitor of HDAC proteins increases the CPP for cocaine, whereas overexpression of HDAC4 in the NAc decreases cocaine preference. These results suggest that chronic cocaine treatment leads to a hyperacetylation at several gene promoters in the NAc and other regions, which increases the reward value for cocaine.

In addition to histone modifications, methylation of the DNA itself is involved in the regulation of gene expression. DNA (cytosine-5) methylation occurs at methyl CpG islands within a gene, and it was once thought to be a permanent way that certain genes are silenced. This silencing occurs during X-chromosome inactivation and several cell-fate determination processes throughout development. Recent studies, however, show that changes in DNA methylation may be more transient and could underlie some of the long-term changes associated with addiction. As with the histone modifications, DNA methylation can occur in response to stimuli experienced during development or early childhood, and this can lead to lasting changes into adulthood. Studies have found that the amount of maternal nurturing behavior in rats is associated with DNA methylation changes in the pups. These different patterns of DNA methylation were associated with the levels of anxiety and maternal behavior of the pups as adults. Therefore, the DNA methylation patterns that are laid down during childhood in response to the environment influence behavior as an adult. As well, women who take drugs while pregnant affect the DNA methylation patterns of the developing fetus. Cocaine use in pregnant women has been associated with changes in DNA methylation at the protein kinase C (PKC) gene in the heart of the fetus. It is likely that there are also methylation changes in the brain that occur with prenatal cocaine exposure that might influence the propensity for addiction in adulthood. It is still unclear whether or not treatment of adult animals with drugs leads to long-term changes in DNA methylation, but this would indeed be a powerful mechanism by which circuits of the brain are altered to allow addiction to develop.

There are many other proteins and cellular alterations that have been associated with addiction. The examples given above highlight some of those that are the most well studied and focus on the regulation of gene expression in response to drug treatment. Some of the genes that are regulated by these

mechanisms include growth factors such as brain-derived neurotrophic factor (*BDNF*), specific ion channels that regulate neuronal excitability, and structural molecules that support cell growth. All of these gene expression changes lead to the altered connections between neurons and changes in neuronal excitability that strengthen or diminish the activity of a given circuit. It is important to understand these molecular changes so that in the future, therapeutic treatments can be developed that will block or reverse the full process of addiction.

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Bruce W. Newton and Robert E. Mrak

CONTENTS

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1. INTRODUCTION

This chapter serves as a brief introduction to some of the basic pathologies encountered by physicians. The chapter is divided into sections dealing with vascular-related pathologies, tumors, infections, congenital malformations, perinatal abnormalities, and several other common pathologies. Some of the specimens shown were collected as early as the 1940s (e.g., the neuroembryologic specimens), before the advent of more sophisticated imaging techniques. These specimens are almost irreplaceable. Since the advent of modern *in utero* imaging techniques, the most severe neurologic deficits can be detected before the baby is born, and this technology now presents parents and physicians with cultural, religious, and moral dilemmas and decisions as to whether to allow the fetus to come to term or to terminate the pregnancy. Other adult specimens were collected more recently and are “routinely” encountered during autopsies or during dissection of cadavers used for gross anatomy or neuroscience courses (e.g., various types of hemorrhages and neoplasms).

2. VASCULAR-RELATED PATHOLOGY

The blood supply to the central nervous system (CNS) is prone to a variety of pathologies. For example, arteries can become stenotic or blocked via atherosclerosis or they can form abnormal connections with veins and produce arteriovenous malformations. Vascular disease can either deprive the CNS of blood supply and produce death of cells or can result in hemorrhage (bleeding) into or around the brain. In either case, the sudden change in mental status is called a *stroke*. In these instances, it must be remembered that the CNS is divided into vascular territories, each of which receives blood from a specific artery. This territorial specificity allows a physician to predict what deficit(s) a patient will have after they have suffered a stroke involving a particular artery. The degree of deficit produced by a stroke is not necessarily correlated with the size of the infarction. For example, a small lesion in the cerebral cortex, depending on the location, may produce only a very minor deficit; yet the same-sized infarct within the brain stem can have devastating consequences that frequently lead to death.

Bleeding can occur into the CNS parenchyma, the ventricular system, and/or the subarachnoid space. Blood may also accumulate on either side of the dura mater and produce a space-occupying lesion that can cause the brain or brain stem to herniate

(squeeze) around dural extensions (i.e., under the falk cerebri or through the incisura of the tentorium cerebelli) or through the foramen magnum. Finally, blood vessels, most commonly arteries, can develop aneurysms that may rupture and bleed into the subarachnoid space.

2.1. Atherosclerosis

Figure 1 shows an example of *atherosclerosis*. This is a common disease that results from fat-rich diets, high blood pressure, diabetes, and certain genetic predispositions. If atherosclerosis is present in the blood supply to the CNS, then it is also seen in other areas of the body. This blockage of blood flow in vessels causes numerous problems throughout the body, including myocardial infarction (heart attack), blindness, and claudication (leg weakness and pain due to poor blood supply). Atherosclerosis in the internal carotid arteries, supplying the brain, can cause death of brain tissue and stroke. Atherosclerosis in intracranial vessels is generally mild and causes dilation; this is different from atherosclerosis seen in vessels outside the brain, where there is narrowing. For this reason, stroke usually results from disease at the origin of the vertebral arteries from the subclavian arteries, at the bifurcation of the common carotid artery into the external and internal carotid arteries (49%

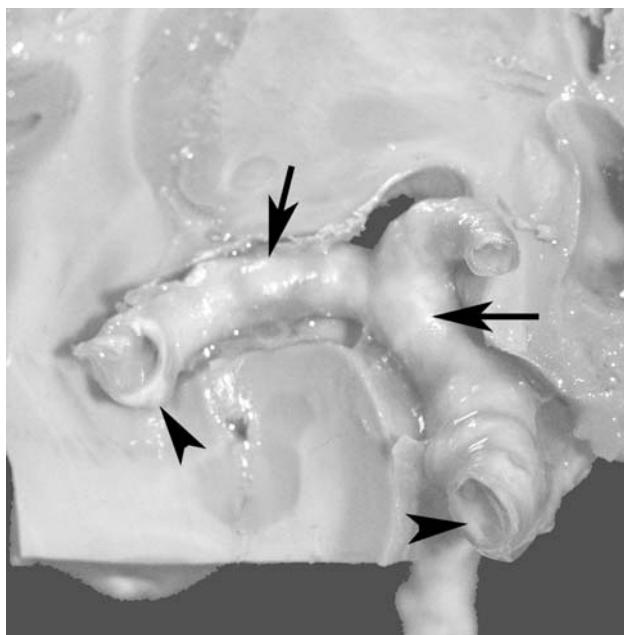


Fig. 1. Abnormal portions of this middle cerebral artery are dilated and show a granular gray discoloration due to atherosclerosis. The sclerotic plaques can be seen beneath the tunica externa (arrows) and in the cut ends of the arteries (arrowheads).

of cases), and at the origins of the middle and anterior cerebral arteries from the internal carotid artery (Fig. 1), rather than from disease of intracranial vessels.

2.2. Aneurysms

Figure 2 shows an example of a *saccular ("berry") aneurysm*. These aneurysms, or outpouchings of the vessel wall, are common (6% to 10% of all adults have them). They are *not* congenital, as they are never found in young children, but they are thought to arise from a congenital defect in the wall of the vessel that only becomes apparent later in life. Cerebral vessels may be particularly susceptible to this as the tunica media of the cerebral vasculature is less well developed than in other organs. This weakness in the tunica media is particularly prominent at branching points. These aneurysms first appear after puberty, suggesting some correlation with the changing hormonal milieu. Occasionally, these aneurysms rupture, causing sudden, massive bleeding into the *subarachnoid space* and stroke. It is not clear why some of these aneurysms rupture and some do not. Survival rates are only 50:50 even if the victim is immediately treated. Death occurs in minutes if the hemorrhage is massive. The most common site of a saccular aneurysm is at the branching point of the internal carotid artery with the posterior communicating artery (41%), closely followed by branch points of the anterior (33%) and middle (20%) cerebral arteries from the internal carotid artery. When the aneurysm bursts, there is the onset of a "thunderclap" headache, with the patient often

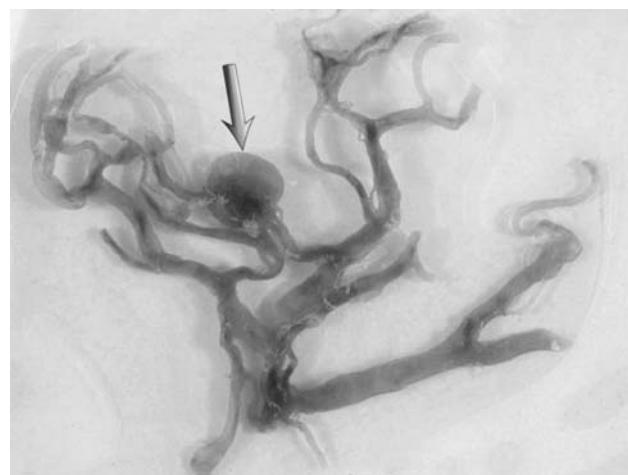


Fig. 2. A saccular (berry) aneurysm (arrow) on one of the major branches of the middle cerebral artery. If a berry aneurysm ruptures, the blood escapes into the subarachnoid space and produces a subarachnoid hemorrhage (see Fig. 19 and Fig. 20A).

stating that they have, all of a sudden, “the worst headache of my life.” Because the arachnoid contains numerous c-fibers (“pain fibers”) and is sensitive to blood, subarachnoid hemorrhage produces excruciating headache. Seizures and arterial spasms in the cerebral vasculature are grave secondary consequences for up to several months after the initial rupture.

2.3. Arteriovenous Malformations

Arteriovenous malformations (AVMs) are abnormal connections between arteries and veins that are present since birth. Many of the cavernous vascular channels do not histologically resemble arteries or veins. Normal arterial pressure causes the veins to dilate and to thicken, causing the lesion to appear to “grow,” but they do not enlarge out of proportion to normal brain growth. Microscopic bleeding is common, and this can cause seizures and headaches. Sudden massive bleeding into the brain can also occur, causing stroke or death. The risk of massive hemorrhage and death increases from middle age onward. These lesions can also siphon blood away from other parts of the brain, causing tissue death and stroke. The brain tissue between the dilated vessels is generally gliotic (scarred) and nonfunctional, making surgical removal of these lesions possible. Figure 3 shows a massive AVM, whereas Fig. 4 shows a smaller AVM.



Fig. 3. A large arteriovenous malformation in a coronal section through the brain. Close examination shows that what appears to be bleeding into the brain is actually blood contained within huge, dilated veins that have expanded from the arterial pressure.

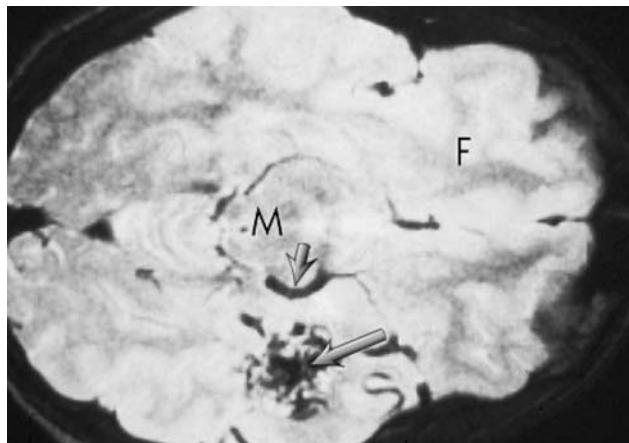


Fig. 4. An arteriovenous malformation (large arrow) is visible in an MRI scan taken in the horizontal plane. The lesion is in the left temporal lobe. Note the large size of the posterior cerebral artery (small arrow) ipsilateral to the lesion. F, frontal lobe; M, midbrain.

2.4. Infarctions

Cerebral infarction is death of brain tissue due to loss of oxygen or nutrients; this is one type of *stroke*. Cerebral infarctions are the third leading cause of death in the United States after heart disease and cancer. Age-adjusted mortality figures indicate a prevalence of 50 to 100 per 100,000 individuals. Factors that increase risk (or chance) of having a stroke include hypertension, cardiac disease, diabetes mellitus, long-term cigarette smoking (especially when combined with oral contraceptives), and hyperlipidemia. More than 85% of strokes occur in the brain above the level of the *tentorium cerebelli* (i.e., *supratentorial* in location). Slightly more than 50% of the supratentorial strokes occur deep inside the brain (e.g., within the putamen or caudate), and one-third involve the cortex and white matter of one or more cerebral lobes. Cerebral infarction most commonly results from reduced blood supply to the brain, and such infarctions are called *ischemic infarctions*. The area infarcted reflects the vascular territory of the occluded vessel. Vascular occlusion may result from *embolization* or from local occlusion. Embolization is movement of a clot or other particulate material from one point to another within the vascular system (e.g., from the heart to a vessel in the brain). The resulting infarction is called an *embolic infarction*. Local occlusion may also occur, as when a *thrombus* (blood clot) forms locally instead of embolizing from elsewhere. An infarction resulting from a local thrombus is called a *thrombotic infarction*. Occasionally, infarction may be caused by an air or fat embolus. Air

emboli can result from surgery on the lungs or internal jugular vein, and fat emboli can result from a bone fracture with release of bone marrow into the vascular system. All of these types are *ischemic*, because they all involve reduced blood flow to the brain.

Ischemia begins when cerebral blood flow is reduced below 30 to 35 mL $100 \text{ g}^{-1} \text{ min}^{-1}$. Infarction occurs at $<20 \text{ mL } 100 \text{ g}^{-1} \text{ min}^{-1}$, and massive infarction occurs at $<15 \text{ mL } 100 \text{ g}^{-1} \text{ min}^{-1}$. Neuronal metabolic changes occur in as little as 30 s after the interruption of blood flow, and a blood flow interruption of 4 to 5 min will cause complete neuronal cell death within the following few hours.

2.4.1. ACUTE INFARCTION

Figure 5, Fig. 6, Fig. 7, Fig. 8, and Fig. 9 show acute (recent) ischemic infarctions. The area of the CNS damaged by the infarction is restricted to the territory supplied by the blocked artery. Infarcts are initially pale in appearance due to the interruption of blood supply. During the first few days, the infarcted area is soft, swollen, and slightly discolored. If blood supply is restored after the initial ischemic event, the infarction may become hemorrhagic (Fig. 9). This may occur, for instance, if the initial embolic material breaks up and the fragments are swept away to more distal sites as blood flow is restored. The blood subsequently seeps through the vessels damaged by ischemia.

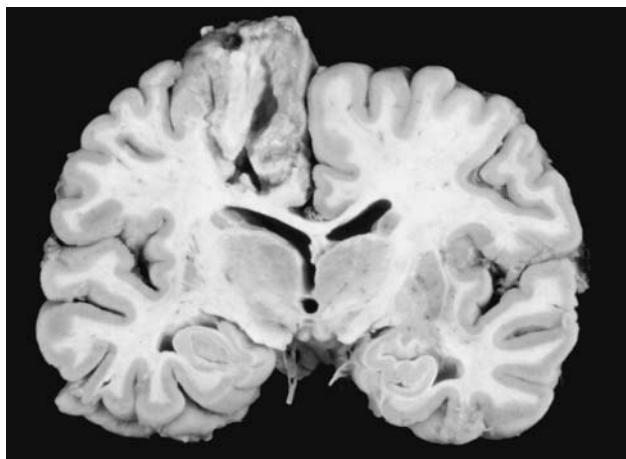


Fig. 5. A coronal section showing the results of an acute infarction of the left anterior cerebral artery. The area of infarction extends from the midline over the vertex of the frontal lobe. The cortex in this arterial distribution is swollen and soft. Cavitation has started to occur. This latter effect is due to death and disruption of axons and consequent softening of the tissue. Had the patient lived, the necrotic material would have eventually been removed (see Fig. 10 and Fig. 11).

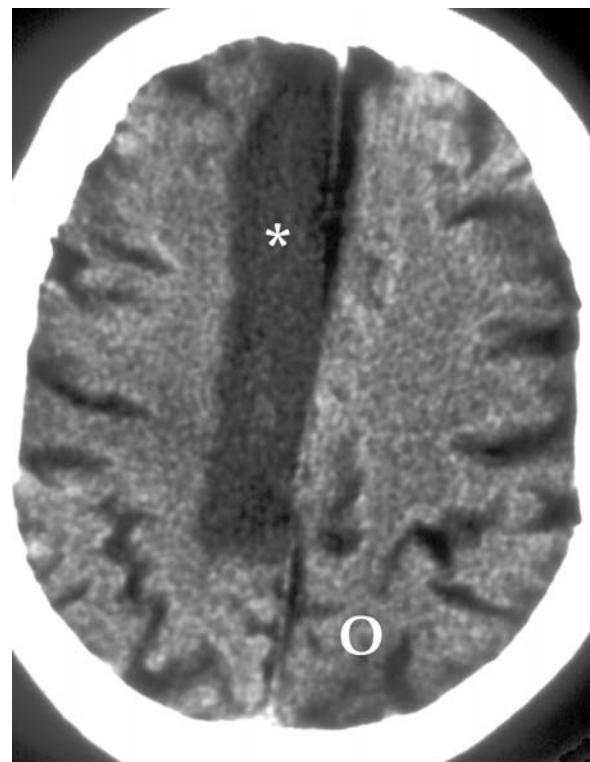


Fig. 6. A CT scan showing a patient with an acute anterior cerebral artery infarct (*homogeneous gray area with asterisk*). The region of ischemia extends from the frontal lobe through the parietal lobe. Note that the occipital lobe (O) is spared as its blood supply comes from the posterior cerebral artery.

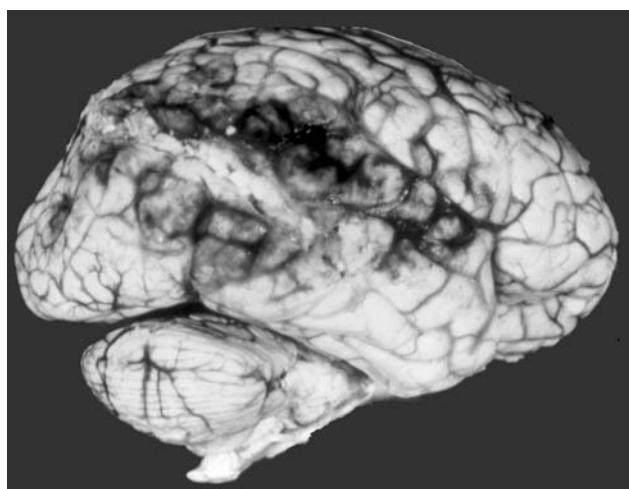


Fig. 7. An acute infarction (*dark mottled region*) involving a branch of the middle cerebral artery that affects portions of the right superior, middle, and inferior temporal gyri and a portion of the inferior parietal lobe. (The horizontal slash through the middle temporal gyrus was caused during removal for autopsy).

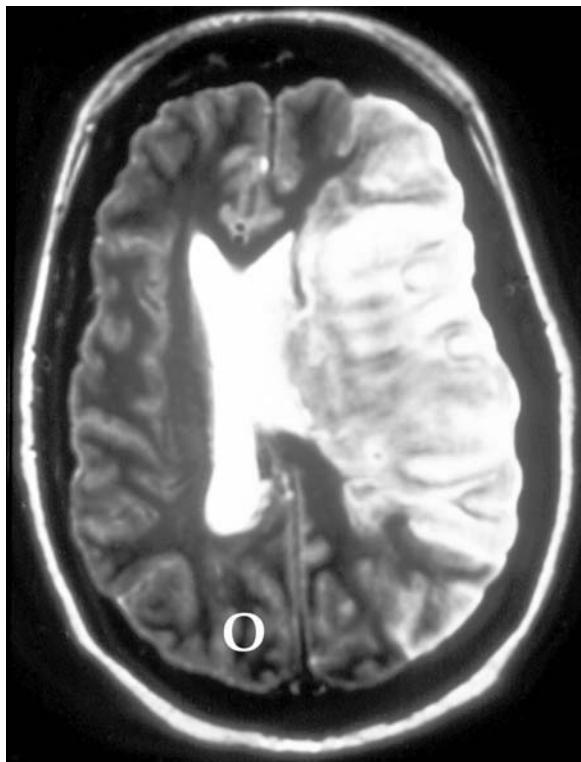


Fig. 8. A massive acute infarction (*white mottled region*) is seen in a T2-weighted MRI scan taken through the horizontal plane. This infarction involves a branch of the left middle cerebral artery and has destroyed a portion of the frontal and parietal lobes. Edema has pushed part of the left lateral ventricle across the midline. O, occipital lobe.

Within an infarction, there are two zones of damage: a central region of complete infarction, which is composed of neurons that have died, and a surrounding *penumbra* that is composed of neurons that have not immediately died but reside in a region of reduced blood supply. The neurons within the penumbra may be saved with appropriate efforts, and this is an area of active research efforts.

2.4.2. CHRONIC INFARCTION

A *chronic infarction* is simply one that is no longer acute (or recent). Over the weeks and months after the onset of the infarction, there is a proliferation of astrocytes and capillaries in the surrounding area in an attempt to promote healing. Necrotic material is removed by phagocytic cells, and eventually a cavity remains. Nonfatal hemorrhages will eventually be resorbed to leave a fluid-filled cavity, similar to that resulting from infarction but brown in color from residual hemosiderin. Figure 10, Fig. 11, and Fig. 12 show various stages of CNS degeneration after a stroke. Neuronal death also leads to degeneration of

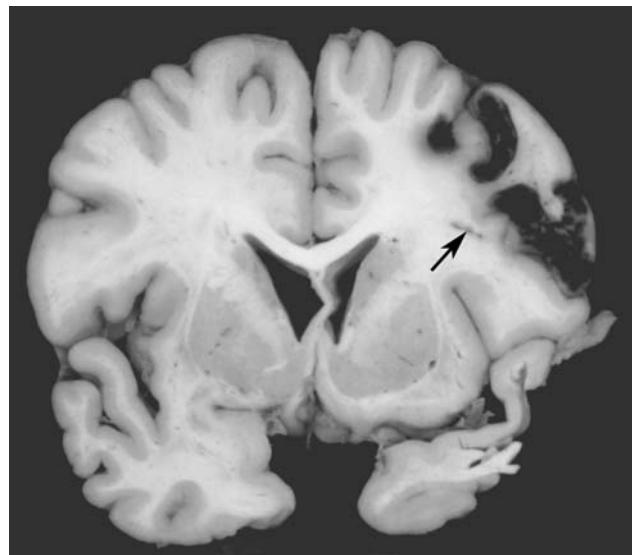


Fig. 9. Strokes can be hemorrhagic. Some branches of the middle cerebral artery have ruptured and blood escaped into the gray matter of the left frontal lobe. Another way in which blood can escape into the parenchyma is during reperfusion after an ischemic attack. The loss of blood flow damages the vessels, and when reperfusion is established, the blood leaks into the parenchyma. A smaller vessel has leaked in the white matter (arrow).

their axons. This can be seen, for example, when upper motor neurons in the primary motor strip die, causing degeneration of the pyramidal tract in the ipsilateral medulla (Fig. 12).



Fig. 10. A stroke in part of the distribution of the left middle cerebral artery has destroyed the portion of the cerebral cortex that contains Wernicke's speech comprehension area. Macrophages have started to remove the dead tissue.

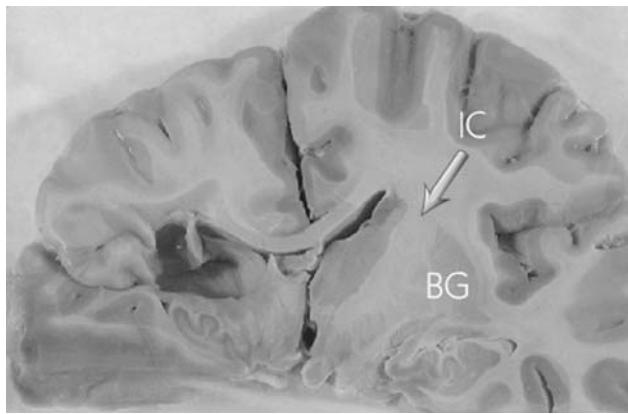


Fig. 11. The coronal section of this brain shows an old infarction involving a branch of the middle cerebral artery. There has been removal of infarcted tissue with formation of a cavity (the cavity has collapsed in the specimen). Compare the right and left halves. The basal ganglia and the internal capsule have been destroyed ipsilateral to the infarction. The insula and cerebral cortex have also been damaged. The normal contralateral basal ganglia (BG) and internal capsule (IC) are indicated.

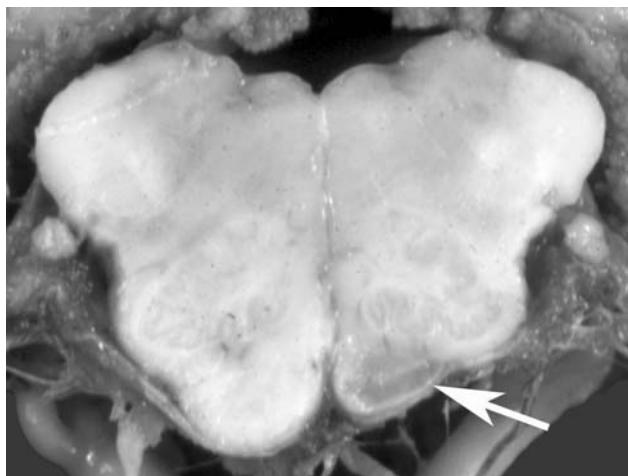


Fig. 12. A cross section of the rostral medulla. The left pyramid (arrow) that contains the ipsilateral corticospinal tract is atrophied due to the loss of the upper motor neuron cell bodies in the primary motor cortex. Compare the size of the atrophied pyramid with the normal-sized contralateral pyramid. Although the stroke occurred in the ipsilateral primary motor cortex and destroyed the upper motor neurons, the loss of their axons leads to atrophy at distant sites.

2.4.3. INTRACEREBRAL HEMORRHAGE CAUSED BY HYPERTENSION

Since the 1950s, when antihypertensive drugs were introduced, the number of strokes due to high blood pressure has decreased. Unfortunately, uncontrolled

hypertension is still problematic. The genetic predisposition for blacks to become hypertensive, when combined with inadequate medical care, causes these individuals to have a higher rate of hypertension, and subsequently strokes, than do other ethnic groups. The combination of hypertension, which exacerbates the development of atherosclerosis, with other risk factors for stroke is particularly dangerous. However, despite the dangers of hypertension, embolic strokes still outnumber hypertensive strokes 4:1. Hypertensive strokes commonly occur in the basal ganglia (Fig. 13) and cerebellum, or pons (Fig. 15).

Hypertensive strokes cause arteries to rupture with subsequent bleeding into the parenchyma. The accumulation of extravascular blood results in a *mass effect* that damages surrounding tissue and also displaces CNS tissue causing cerebral or cerebellar herniations.

Common sites for hypertensive hemorrhage are the basal ganglia (Fig. 13; “ganglionic” hemorrhage) and the brain stem. Basal ganglia hemorrhages arise from bleeding of the *lenticulostriate arteries* that supply the basal ganglia. The lenticulostriate arteries have their origin from proximal vasculature near the *circle of Willis* and are subjected to higher arterial pressure than are other cerebral end-arteries, most of which arise from distal vasculature. Besides bleeding, these arteries can also “hollow out” large perivascular spaces due to their tortuosity, constant pulsing with arterial

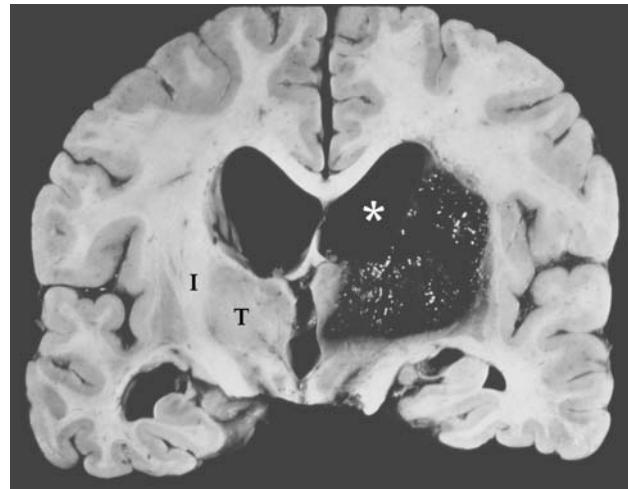


Fig. 13. A hemorrhagic stroke has destroyed the left thalamus and internal capsule. In this instance, the blood is contained within the parenchyma and has not ruptured into the ventricular system (asterisk). This stroke was caused by uncontrolled hypertension. The contralateral thalamus (T) and internal capsule (I) are indicated (see also Fig. 14).

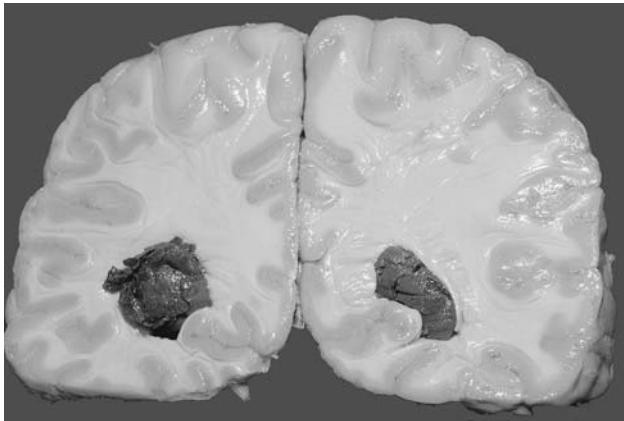


Fig. 14. A hemorrhagic stroke occurred at the level of the basal ganglia. This section, through the occipital lobes, shows that the blood has ruptured into and filled the ventricular system. Hemorrhagic strokes that burst into the ventricular system have a much poorer prognosis for the patient than do those that remain in the brain parenchyma. (The slight swelling of the gray matter is an artifact of fixation and not edema due to the stroke.)

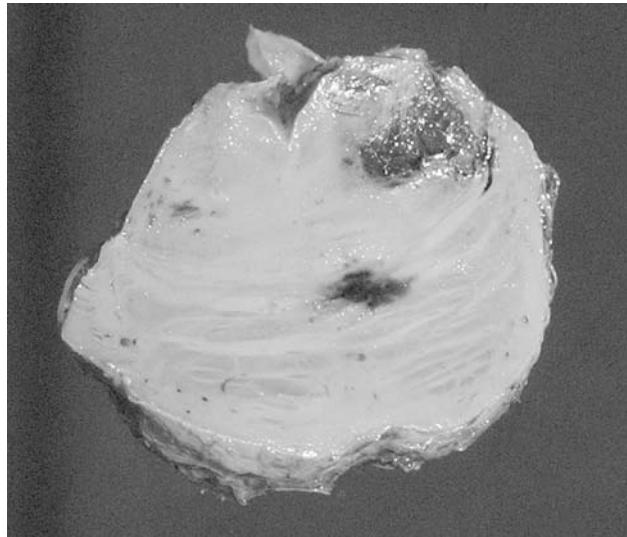


Fig. 16. This specimen shows Duret hemorrhages in the rostral pons. Most hemorrhages of this type have a unique pathogenesis. A supratentorial mass lesion (tumor, hematoma, etc.) displaces (herniates) the brain stem downward, while the basilar artery—which is tethered above by the circle of Willis—does not move. As a result, the small perforating branches of the basilar artery are broken, resulting in these small hemorrhages that are often the cause of death in such patients.

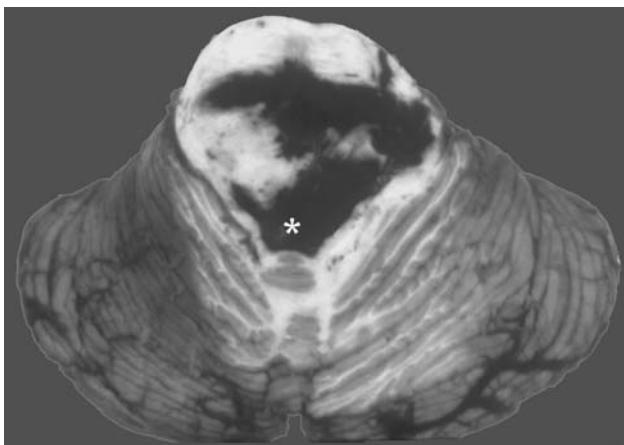


Fig. 15. Small hemorrhagic strokes that occur in the brain stem are much more devastating than those of the same size that occur in the cortex. In this specimen, hypertension has caused a massive pontine stroke that ruptured into the fourth ventricle (asterisk).

pressure, and microscopic bleeding, resulting in miniature areas of infarction that are called *lacunae*. Generally, the signs and symptoms associated with this type of infarction result not from damage to the basal ganglia but from damage to the genu and posterior limb of the internal capsule that carry the corticobulbar and corticospinal upper motor neuron axons. Therefore, the patient typically presents with upper motor neuron-type signs versus basal ganglia

signs. Significantly, any hemorrhage that ruptures into the lateral ventricle (Fig. 14), spreads through the ventricular system, and enters the subarachnoid space makes a fatal outcome much more likely.

A special type of hemorrhage is caused by an intracerebral space occupying lesion and the resulting downward herniations (Fig. 16). A supratentorial tumor, hematoma, and so forth, can cause the brain stem to herniate downward, while the basilar artery—which is tethered above by the circle of Willis—cannot follow the downward movement. As a result, the small perforating branches of the basilar artery are broken, resulting in small *Duret* hemorrhages in the pons. Duret hemorrhages are often the cause of death in such patients.

2.5. Contusions

Tissue death can also result from trauma, and such lesions are called *contusions*. Contusions share many gross and microscopic features with infarction. The two are distinguished by their locations and by the clinical history. Head injury is a major cause of death in young adults between the ages of 15 and 24 years. Traumatic brain injury is 3 to 4 times more frequent in males than in females: motorized vehicle accidents

and personal violence are major contributors. In the United States, it is estimated that a head trauma occurs every 7 s with a death every 5 min. More than 40% of the deaths occur at the scene of the accident and another 20% die in the emergency room. Survivors of serious closed-head injuries may have psychosocial difficulties, may not be able to return to work, or may move to jobs that are less sophisticated than those held prior to the head injury. Less than 20% ever become financially independent.

The majority of contusions occur in the frontal poles, the orbital portion of the frontal lobes, and the temporal poles. These regions of the brain are more susceptible to injury caused by sudden deceleration. Figure 17 shows a contusion to the frontal lobes. The edema that results from a contusion is dangerous and can cause a mid-line shift of brain matter (Fig. 18) and/or a tentorial herniation. Therefore, a small window of time exists for the emergency personnel to get the patient to an emergency room where a physician can take immediate measures to reduce brain swelling. The edema results in an increased intracranial pressure that consequently causes a decrease in blood flow to the cerebrum. The decrease in cerebral perfusion results in more ischemic injury that leads to a vicious circle of more swelling and decreasing blood flow.



Fig. 17. This CT scan shows hemorrhagic contusions in both frontal lobes. CT is best for imaging fresh blood, which is revealed as a hyperintense (white) signal. Note that the hemorrhaging blood is surrounded by a halo of edema that is revealed by the dark gray signal. O, occipital lobe.

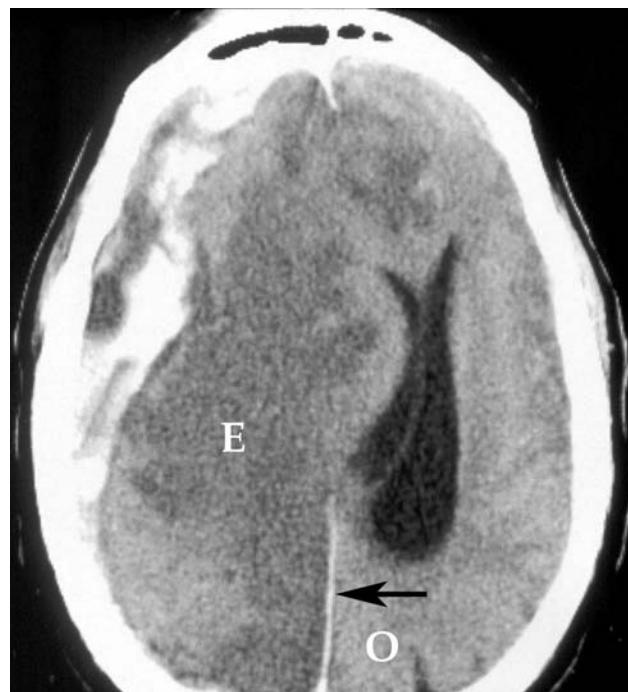


Fig. 18. This CT scan shows the effects of edema. A large, acute subdural hematoma (white mottled crescentic shape) is present on the right side of the calvaria. There is massive edema (E) in the ipsilateral brain that has created a midline shift of the brain beneath the falx cerebri (arrow). Note the entire right lateral ventricle has been pushed across the midline. Frontal sinuses (dark areas) can be seen in the frontal bone. O, occipital lobe.

Concussions are less serious forms of injury than are contusions. Contusions involve overt brain damage with hemorrhage and edema. Contusions usually cause a loss of consciousness, and if this lasts longer than 6 h, it is assumed that brain injury has occurred. In contrast, concussions show no gross structural brain damage. However, repeated concussions are deleterious in their accumulative effect, and athletes prone to multiple concussions, for example, boxers (*dementia pugilistica*), and to a lesser extent football and soccer players, have shown signs of dementia and parkinsonian syndrome (a movement disorder) as they age.

2.6. Extracerebral Hemorrhages

Intracranial bleeding can occur outside of the CNS parenchyma. Hemorrhages may occur between the arachnoid and the brain (*subarachnoid hemorrhage*), between the arachnoid and the dura (*subdural hemorrhage*), or between the dura and the skull (*epidural hemorrhage*). The brain atrophies (shrinks) with

normal aging, opening a space between the arachnoid and the dura into which subdural hemorrhage may occur. There is not normally a space between the dura mater and the skull, but pressure from a broken artery can force blood into this *potential space* to produce epidural hemorrhage. Likewise, blood can be forced into the potential space between the dura mater and arachnoid to produce a subdural hemorrhage. The latter two types of bleeding produce masses of blood (*hematomas*) that put pressure on the brain causing a “mass effect” that results in mid-line shifts of neural structures and/or CNS herniations. These three categories are not just fine anatomic distinctions, but rather they are caused by different factors and result in different clinical presentations.

2.6.1. SUBARACHNOID HEMORRHAGE

Subarachnoid hemorrhage is common (Fig. 19 and Fig. 20A), and if the amount of bleeding is small, it is generally not clinically significant. An exception is massive subarachnoid hemorrhage resulting from the rupture of a berry aneurysm of the type shown earlier (Fig. 2). There are about 26,000 cases of massive subarachnoid hemorrhage in the United States per year. Hypertension, which causes the development of saccular aneurysms, is a contributing cause of subarachnoid hemorrhage, as is *Marfan syndrome* and *sickle-cell disease*. The bleeding may destroy vital structures or divert blood flow, causing infarctions, and either of these effects can

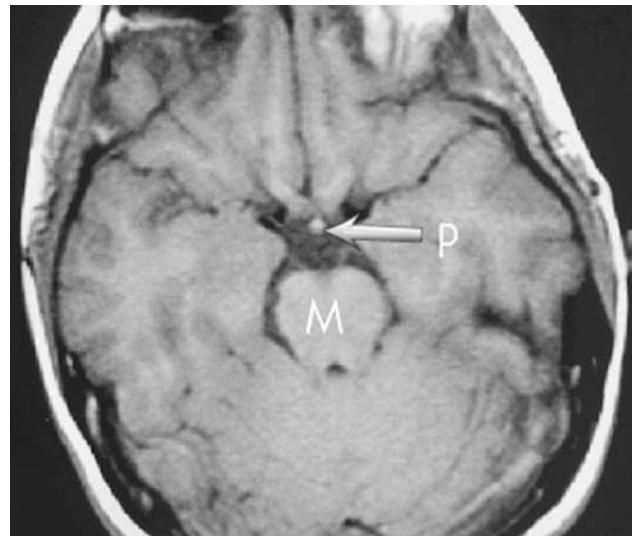
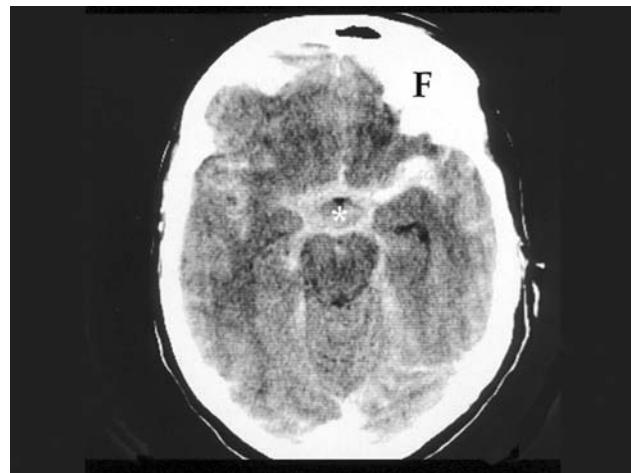


Fig. 20. (A) A CT scan of a subarachnoid hemorrhage. On CT, a subarachnoid hemorrhage is best imaged by looking at a plane of section through the midbrain. At this level, the subarachnoid space has a five-pointed star-like shape as it surrounds the pituitary stalk (asterisk) and midbrain. If blood is present, the CT will reveal a bright subarachnoid space. F, orbital plate of frontal bone. (B) An MRI scan through a normal brain at nearly the same plane of section seen in (A). Note how the subarachnoid space around the midbrain (M) and pituitary stalk (P) is dark. In a CT scan, if the subarachnoid space is filled with blood, the signal is bright (see A).

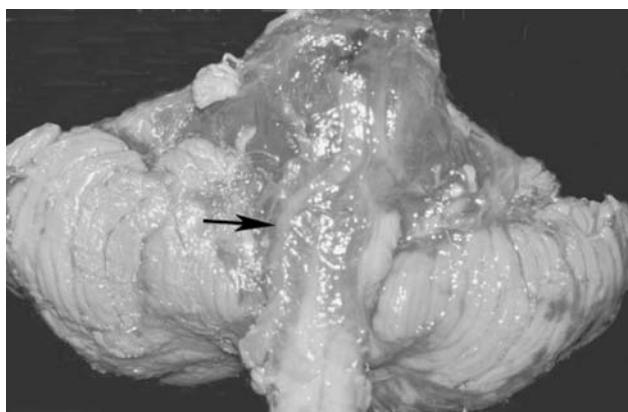


Fig. 19. A subarachnoid hemorrhage covers the brain stem and part of the cerebellum. Note the diffuse layer of blood beneath the arachnoid, without formation of a mass (hematoma). The amount of blood present in this specimen is not sufficient to cause any space-occupying effect. The vertebral artery can be seen (arrow).

cause death. Slow bleeding into the subarachnoid space can eventually clog the arachnoid granulations that shunt CSF into the venous dural sinuses. If this occurs, enlargement of the cerebral ventricles (*hydrocephalus*) can develop.

2.6.2. SUBDURAL HEMATOMA

A hematoma is a large collection of blood that forms a space-occupying mass. Subdural hematomas result from tearing of *bridging veins* that reach from the brain to the arachnoid. The blood accumulates between the arachnoid and the overlying dura mater. These are especially common in the elderly, in whom the bridging veins may already be stretched from shrinkage of the brain (cerebral atrophy). Because the torn vessels are veins, the bleeding often develops slowly.

Acute subdural hematomas (Fig. 21 and Fig. 22) are often associated with some other type of head injury. Almost one-half of the patients that have an acute subdural hematoma are unconscious when they are seen in the emergency room. If the patient does not go into a coma from the associated brain trauma, they may become symptomatic within 3 days. On a computed tomography (CT) scan, an acute subdural hematoma forms a crescent-shaped hyperdense mass that deforms the brain. The bleeding is restricted to one side of the cranium by the falx cerebri. If the acute subdural hematoma is causing a mass effect, it needs to be evacuated. Unlike epidural hematomas, which are caused by arterial bleeding and which continue to expand, venous bleeding from subdural hematomas is usually arrested by rising intracranial pressure.

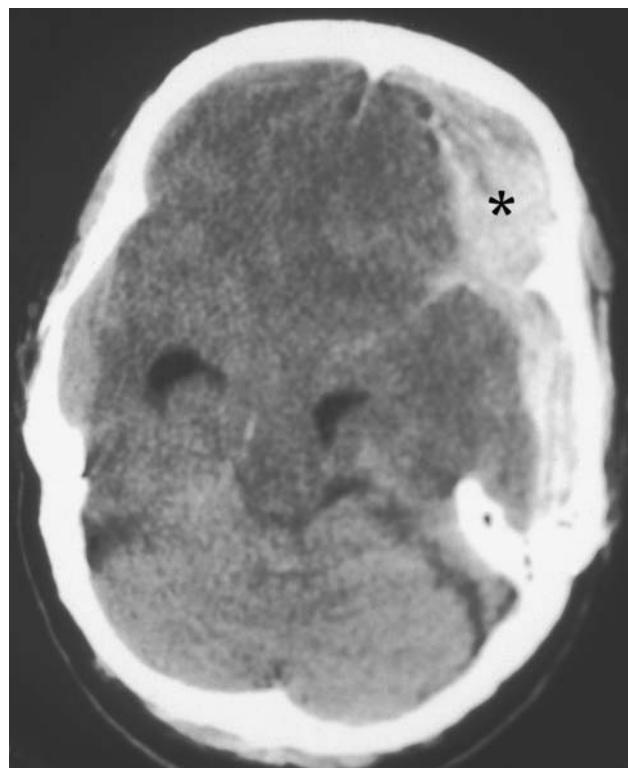


Fig. 22. A CT scan of an acute subdural hematoma (*asterisk*) over the left frontal and parietal lobes. A subdural hematoma presents with a crescentic shape that is restricted to one side of the brain. With an acute subdural hematoma, the blood appears hyperdense in relation to the brain.

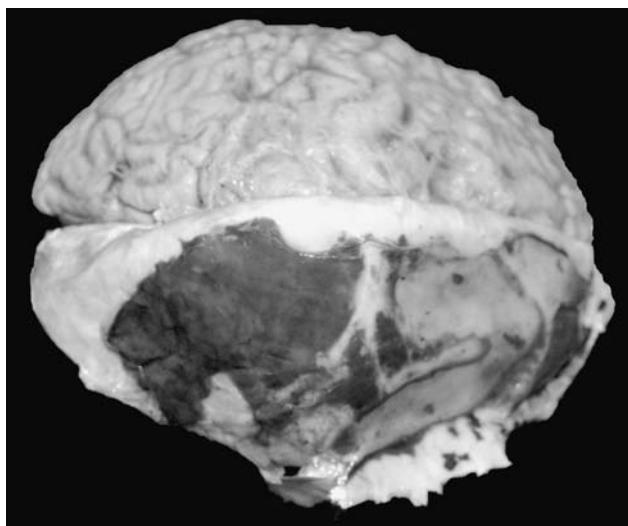


Fig. 21. A specimen of an acute subdural hematoma. The dura from the left side of the brain has been folded back over the right cerebral hemisphere to show the underside of the dura mater and the fresh (acute) blood. Subdural hematomas develop slowly because the source of the blood is from the low-pressure venous system.

Old (chronic) subdural hematomas (Fig. 23 and Fig. 24) may become symptomatic weeks or more after the initial hemorrhage. This is generally due to repeated episodes of bleeding and slow enlargement of the hematoma. The patient may show slowness of thought, confusion, apathy, and drowsiness, symptoms that may be mistaken for other neurologic problems. Chronic subdural hematomas are more likely to occur after the age of 50. In about half of the cases, the head trauma was so mild that it is not remarked upon or remembered. Anticoagulant drugs may contribute to the formation of these hematomas by making recurrent bleeding more likely. Over the weeks after the hemorrhage, the clot becomes slowly encapsulated by fibrous tissue that grows out from the dura. Neuroradiologic imaging shows an isodense or hypodense mass that deforms the surface of the brain.

2.6.3. EPIDURAL HEMATOMA

Approximately 85% of epidural hematomas (Fig. 25) result from tearing of the middle

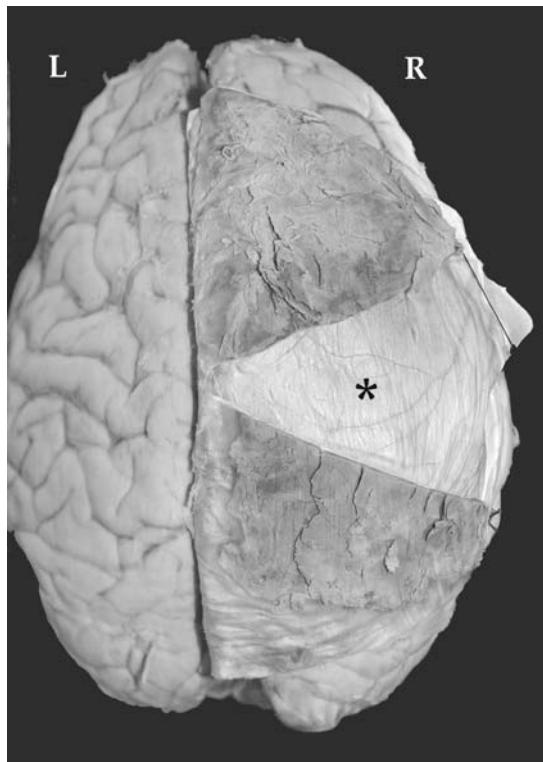


Fig. 23. A specimen of an old (chronic) subdural hematoma. The dura mater has been folded back from the left (L) cerebral hemisphere over the right (R) hemisphere. A wedge has been removed to reveal the bright periosteal surface of the dura mater (asterisk) covering the right cerebral hemisphere. Note the old, pale-gray blood on the undersurface of the dura mater on the left side that has been “organized” (i.e., the blood has been broken down and removed by macrophages) and has been replaced by proliferating small blood vessels and collagen.

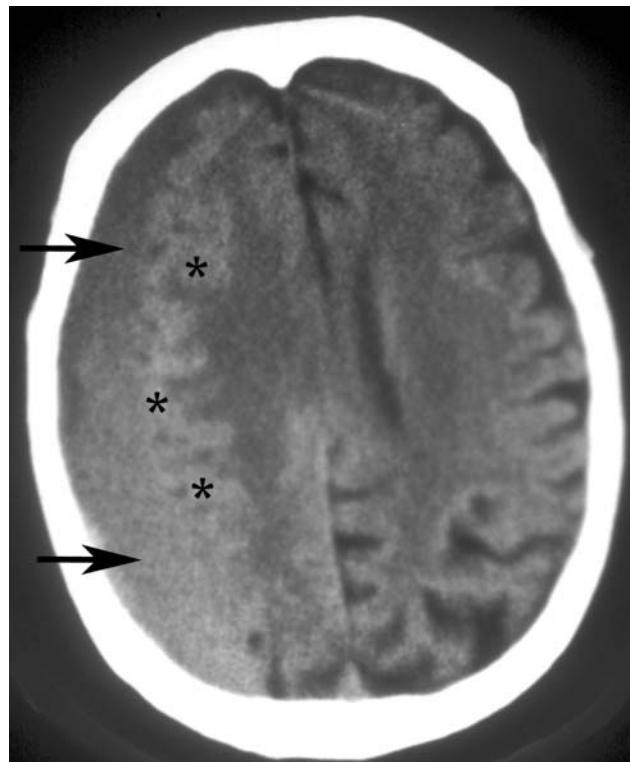


Fig. 24. A CT scan of a chronic subdural hematoma (arrow) that covers the entire right cerebral hemisphere. Note how a subdural hematoma forms a crescentic shape on a CT scan. The blood in a chronic subdural hematoma is less bright than the signal made by fresh blood in an acute subdural hematoma (see Fig. 22). The brighter convoluted surface of the cerebral cortex can be seen beneath the hematoma (asterisks). The left cerebrum shows evidence of atrophy, as a dark subarachnoid space is evident beneath the calvaria (bright white) contralateral to the subdural hematoma.

meningeal artery as a consequence of skull fracture (see Fig. 26 in Chapter 2). This type of injury requires considerable force, and patients with epidural hematomas, unlike patients with subdural hematomas, usually have an obvious and recent history of trauma. The lesion develops rapidly because arterial pressure is driving the bleeding, and the hematoma accumulates rapidly between the skull and the periosteal layer of the dura mater. Epidural hematomas constitute a medical emergency as almost 100% of these patients will die if untreated. Even with treatment—removal of the clot and ligation of the bleeding artery—there is a 30% death rate. The mass effect of the hematoma results in increased intracranial pressure that results in herniation of the uncus of the temporal lobe over the edge of the *incisura of the tentorium*

cerebelli (uncal herniation). The herniated tissue stretches the third nerve, producing a third cranial nerve palsy (i.e., the eye on that side is deviated “down and out” and the pupil will be enlarged due to the removal of parasympathetic tone to the constrictor pupillae muscle). Downward herniation of the brain stem can cause Duret hemorrhages of the pons (Fig. 16), and downward herniation of the cerebellar tonsils into the foramen magnum can compress the medulla with fatal results.

Epidural hematomas are generally lens-shaped, large, and confined to the middle cranial fossa as they cannot expand across suture lines. The body has no mechanism to remove blood from the artificially created epidural space, and if the clot is not removed, death occurs within a few days.

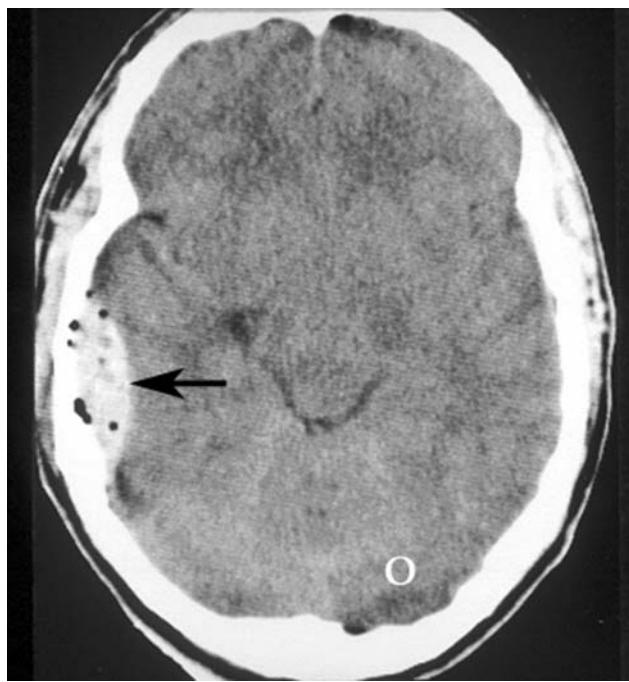


Fig. 25. A CT scan revealing a developing epidural hematoma (arrow) that covers part of the right cerebral cortex. Note that the fresh blood has a much brighter CT signal than the blood seen in a chronic subdural hematoma (see Fig. 24). The black circles in the epidural hematoma represent air leaking in from the skull fracture. Epidural hematomas most commonly result from tears in the middle meningeal artery. Thus, this is the most common position to image an epidural hematoma. Epidural hematomas present as a lens-shaped lesion on a CT scan and do not cross suture lines. O, occipital lobe.

The symptoms produced by tumors depend more upon *where in the brain* they are than upon *what type of tumor* they are. Tumors that affect primary regions of the brain dealing with motor or sensory functions cause symptoms much earlier than those that are growing in “silent” cortical association areas. If a tumor is enlarging rapidly, or there is massive edema, then the symptoms present much earlier than a tumor that grows slowly. Headache occurs in 50% to 60% of patients with tumors, and seizures occur as a presenting symptom in 30%.

3.1. Tumors That Are Space-Occupying, Noninfiltrative, and Arise Outside of the CNS

Some tumors have a very circumscribed border and do not infiltrate into the surrounding neural tissue, that is, they remain cohesive (all of the cells cling to one another). Within the cranium, the most common noninfiltrative tumor that arises outside of the CNS is *meningioma* (Fig. 26). These usually benign tumors arise from the dura mater and grow very slowly. As they expand in size, they slowly compress the CNS tissue beneath them. Some meningiomas can grow to extraordinary sizes (as large as an orange or larger) before a patient has symptoms that cause them to come to a physician—indicating the remarkable capacity for the brain to accommodate a space-occupying lesion if the expansion is slow enough.

The most common site for a meningioma is the *falk cerebri* (25%). More than 85% of these usually

3. TUMORS

Most tumors are space-occupying lesions that can eventually cause herniation of brain tissue as they expand. Metastatic (“coming from elsewhere”) tumors originate from tumor cells or small clusters of tumor cells that arrive via the bloodstream (i.e., they embolize) from peripheral organs, and then grow into larger masses in the brain. Tumors such as meningiomas can originate from the dura or from the periosteum of the skull base and press against the brain causing a mass effect. Other tumors are “primary” in the CNS; that is, they arise from intrinsic brain cells, most frequently from the various glial cells. There are approximately 24,000 primary brain tumors and an equal number of metastatic tumors diagnosed in the United States each year. The frequency of primary tumors is estimated to be 7 to 8 per 100,000 population. Gliomas (tumors of glial cells) constitute nearly 60% of the primary tumors.

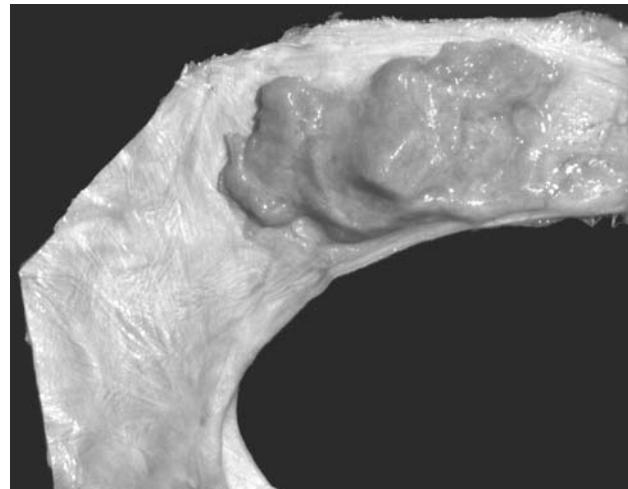


Fig. 26. An isolated falk cerebri specimen with a meningioma. This meningioma has calcified, an occurrence that can happen as these tumors age. About 25% of meningiomas arise from the falk cerebri, which makes this site the most common location for meningiomas.

benign tumors arise in the anterior cranial fossa. Meningiomas account for 20% of all intracranial tumors and are twice as frequent in females as in males. Although meningiomas grow slowly, their growth never stops and eventually leads to the death of the patient due to the compression of vital CNS structures. Surgical removal is generally curative.

Examples of malignant tumors that are contained within the cranial vault but outside of the CNS include tumors arising from cartilage (chondrosarcoma) and from bone (osteosarcoma). A second example of a benign, noninfiltrative tumor that arises outside the brain parenchyma is a *schwannoma* (not shown). Schwannomas arise from the Schwann cells that produce the myelin on peripheral nerves. Schwannomas are smooth, well-defined, and noninvasive. These tumors can arise from any nerve in the body, but intracranial schwannomas predominately arise from the vestibular portion of the vestibulocochlear nerve. These particular schwannomas are also called acoustic “neuromas,” even though the tumor is *not* composed of neurons. Small (<2 cm in diameter) schwannomas generally can be surgically resected with preservation of facial nerve function (recall that the seventh cranial nerve courses with the eighth cranial nerve in the internal acoustic meatus) and of any auditory and vestibular function that was not destroyed by the tumor growth. Other common intracranial sites for schwannomas are the trigeminal and facial nerves.

3.2. Metastatic Cancer

Some space-occupying, noninfiltrative lesions consist of cancerous cells that have metastasized from a primary tumor elsewhere in the body (Fig. 27). In patients with systemic cancer, 25% will develop brain *metastases*. The most common sites of origin for metastatic tumors found in the brain parenchyma include lung (46%), breast (13%), colon (9%), and marrow (leukemia) (7%). Other than leukemia (Fig. 28), all of the metastatic cancers are epithelial in origin. Small metastases seem to favor the gray/white junction. The metastases may be single or multiple (*miliary*) with a little less than half of the metastases being single in nature. Metastatic brain tumors can often be completely removed surgically, and this improves quality of life in these patients. However, they generally die from the original (non-CNS) tumor growth.

While these epithelium-derived tumors will grow in the brain, the cells remain cohesive and will not infiltrate brain tissue as single cells. Metastases are thus sharply demarcated lesions, in contrast with primary brain tumors such as astrocytomas and glioblastomas,

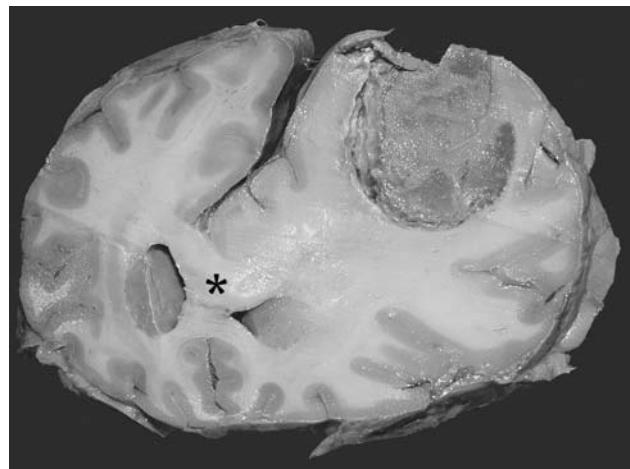


Fig. 27. This coronal section of the brain shows a large metastatic tumor from lung located in the frontal lobe. This metastasis is space-occupying and has severely compressed the brain matter around it. The lesion is dorsal to the rostrum of the corpus callosum (asterisk).

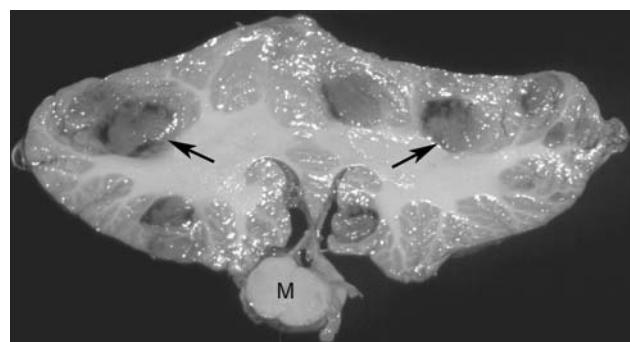


Fig. 28. This horizontal section through the cerebellum reveals multiple foci of metastatic leukemia (arrows). Note how the lesions are found in the gray matter versus the white matter, as the gray matter is more vascular. M, medulla.

which do not form distinct borders. Sometimes, metastatic tumors become necrotic, in which case they may grossly resemble abscesses except that there is no fibrous wall surrounding them.

3.3. Infiltrative Tumors of CNS Origin

Malignant CNS tumors, unlike metastatic tumors, infiltrate surrounding brain tissue making them generally impossible to remove completely. Figure 29, Fig. 30, and Fig. 31 show examples of malignant neoplasms that arise from within the CNS. There are about 8500 new cases of these primary CNS neoplasms in the United States each year. The majority of these tumors arise from glial cells and are collectively called *gliomas*. Most gliomas invade surrounding brain and eventually

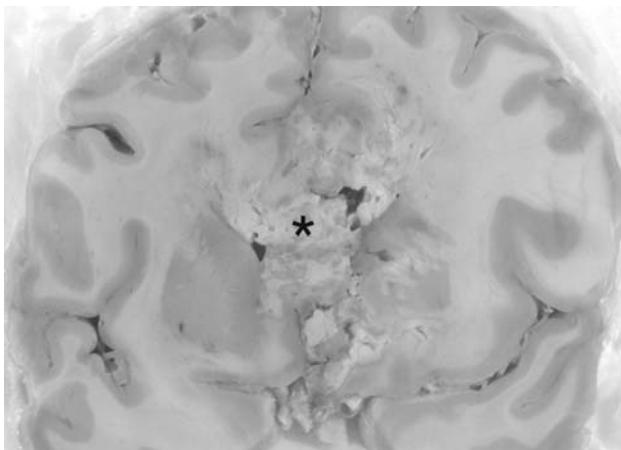


Fig. 29. A coronal section through a brain with a glioblastoma (a high-grade malignant glial tumor). Note the irregular necrosis and hemorrhage that makes the tumor stand out grossly. Glioblastomas are microscopically, however, poorly demarcated, in contrast with metastatic tumors (see Fig. 27 and Fig. 28). What you think is the edge of the tumor is actually the edge of the necrosis and hemorrhage. Note how the tumor tends to follow white matter pathways and is spreading into the contralateral hemisphere via the axonal pathways of the corpus callosum (asterisk).

kill the patient. The borders of these tumors are consequently not smooth or well-defined. In fact, it is generally impossible to grossly define exactly where the tumor edge is or is not. The more malignant (faster-growing) types show areas of *necrosis* and hemorrhage, which may impart a deceptive appearance of definition to the tumor's borders.

Gliomas are divided into different types that correspond with the types of glial cells from which they arise. The vast majority are *astrocytomas* (Fig. 29 and Fig. 30), with *ependymomas* (Fig. 31) and *oligodendrogliomas* comprising <6% of the total. A highly malignant (fast-growing) glioma, regardless of glial cell origin, is called a *glioblastoma* (Fig. 29 and Fig. 30). A common childhood tumor is *medulloblastoma*, a highly malignant tumor of "primitive" (i.e., featureless, or undifferentiated) neuroectodermal cells that arises from the roof of the fourth ventricle. A tumor that starts as "low grade" (i.e., not highly malignant) will sometimes dedifferentiate into a "high grade" tumor whose growth becomes increasingly aggressive.

On contrast-enhanced CT or magnetic resonance imaging (MRI) scans (Fig. 30), infiltrative tumors appear as hyperintense areas due to breakdown of the *blood-brain barrier* and leakage of the contrast material from capillaries into the tumor. Glioblastomas are rapidly growing and outstrip their blood

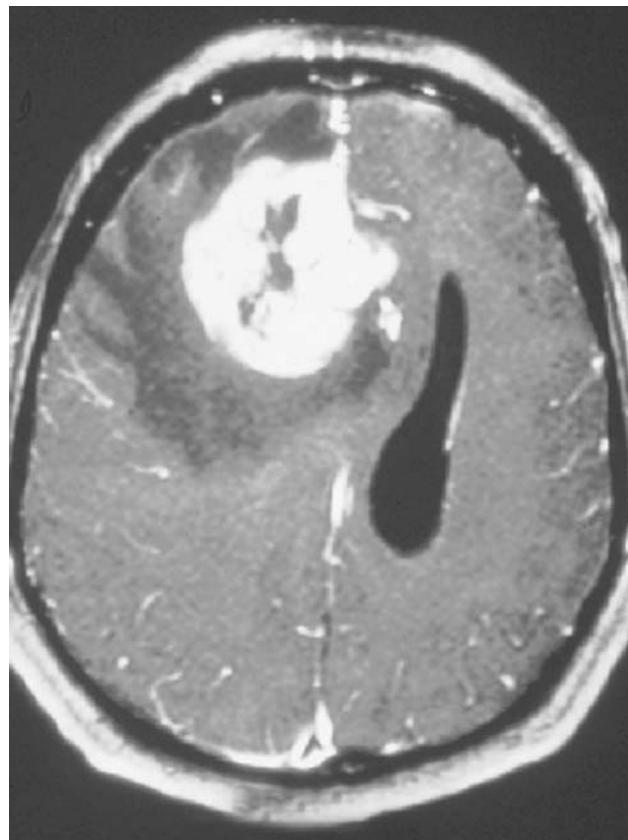


Fig. 30. This MRI scan shows the typical appearance of a glioblastoma within the CNS as visualized with contrast. Note how the gadolinium contrast agent has leaked out of the disrupted blood-brain barrier and demarcated the position of this "ring-enhancing" lesion. This tumor has a necrotic core that appears hypodense. Note the edema (*dark border*), which surrounds the tumor. The ipsilateral lateral ventricle has been obliterated by the increased intracranial pressure generated by this space-occupying mass.

supply resulting in central areas of tumor necrosis. Enhancement is not seen in these necrotic areas (because there is no blood supply to carry the enhancement dye there), and so a characteristic pattern of *ring-enhancement* is seen on imaging (Fig. 30). The rapidly growing tumor also induces a surrounding zone of edema.

Infiltrative tumors cannot be entirely resected, but surgery can be used to debulk the tumor in order to slow the growth, to decrease the space-occupying effect, and to decrease the resultant increased intracranial pressure. Unfortunately, these tumors will all eventually regrow and are ultimately lethal; survival ranges from 14 to 40 weeks. The individual symptoms of gliomas are nonspecific and include headache, vomiting (especially if the tumor is infratentorial),

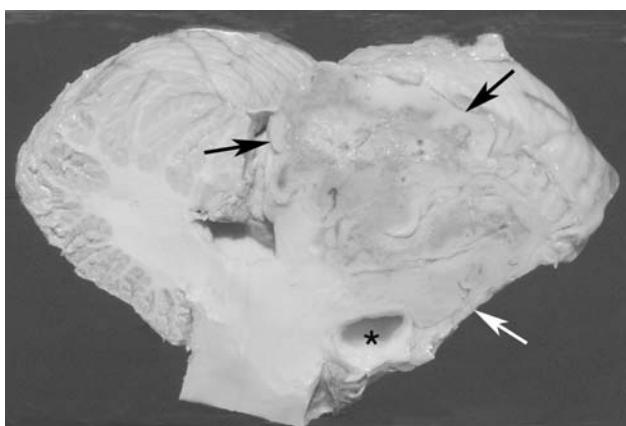


Fig. 31. This cerebellar specimen shows an ependymoblastoma (*bounded by arrows*) that arose from the ependymal lining of the fourth ventricle. This neoplasm has infiltrated the cerebellum and has partially obliterated the fourth ventricle. A necrotic region (*asterisk*) is clearly visible.

seizures, and eventually slow changes in mental function. A pattern of worsening symptoms may suggest a tumor.

Ependymomas are primarily tumors of childhood and young adults (Fig. 31). These tumors originate from the cells that line the ventricular system. In children, these devastating tumors are often found in the fourth ventricle and invade the cerebellum. Symptoms include cerebellar dysfunction, nausea, and vomiting due to brain stem dysfunction and headaches caused by increased CSF pressure due to the formation of a *noncommunicating hydrocephalus*.

3.4. Other CNS Tumors

There are several other types of common CNS tumors that should be mentioned. One might expect that neurons would also give rise to tumors, but *neurocytomas* and related tumors are uncommon.

Tumors of the *pineal gland* can occur and account for 1% of all intracranial tumors seen in the United States. The pineal gland has a complex structure that is composed of glandular tissue, glial cells, endothelial cells, and sympathetic nerve endings; and tumors can arise from all of these components. Unfortunately, the pineal gland may harbor primitive germ cells that can become malignant and account for one-third of all pineal tumors. Gliomas account for another third of pineal tumors, and pineal cell tumors account for about 22% of pineal tumors. Males have a much greater chance of having a pineal tumor than do females.

Pineal tumors become symptomatic when they enlarge and press on the midbrain. This closes the

cerebral aqueduct and results in a noncommunicating hydrocephalus with the resultant increased intracranial pressure. Compression of the midbrain causes *Parinaud syndrome* where there is a paralysis of upward gaze. Endocrine disorders also occur but are rare; and those symptoms depend on whether the tumor is composed of pinealocytes or if the tumor destroys the pinealocytes.

Pituitary tumors are common and may even be incidental findings at autopsy. The frequency ranges from 3% to 27%. Many *microadenomas* (<1 cm in diameter) of the pituitary are asymptomatic. Most pituitary tumors arise in the *anterior pituitary*; tumors of the posterior pituitary are extremely rare. Once a pituitary adenoma is large enough, it begins to compress the optic chiasm, and a *bitemporal hemianopsia* results. This visual defect, along with endocrine dysfunction, is one of the primary diagnostic features of a pituitary tumor. Pituitary adenomas can become enormous and erode the *sella turcica* of the sphenoid bone to compress surrounding structures as they enlarge. Cranial nerves within the cavernous sinus or in its dural wall can be affected. Endocrine dysfunction will vary depending on which cellular type comprises the adenoma: *galactorrhea* (excess prolactin production), *acromegaly* (excess growth hormone production), and *Cushing disease* (hypersecretion of ACTH) are the most common. If a tumor destroys the anterior pituitary, then symptoms of hypopituitarism occur.

The perfection of the trans-sphenoid surgical approach to the sella turcica, combined with microsurgical techniques, over the past three decades has allowed neurosurgeons to successfully remove pituitary adenomas. With this approach, the mucosa of the nasal cavity is incised and raised, and the microsurgical instrument is passed beneath the mucosa and into and through the sphenoid sinus. The thin bone of the anterior aspect of the sella turcica, which forms the posterior midline border of the sphenoid sinus, is penetrated and the adenoma resected. Now that replacement hormones have been developed, the lost anterior pituitary function can be fairly well managed.

4. INFECTIONS

Although many infections do not produce space-occupying lesions, some may produce lesions that are space-occupying, for example, *abscess* (Fig. 32 and Fig. 33). Abscesses are sharply demarcated nodules with *central necrosis* and a surrounding reaction.

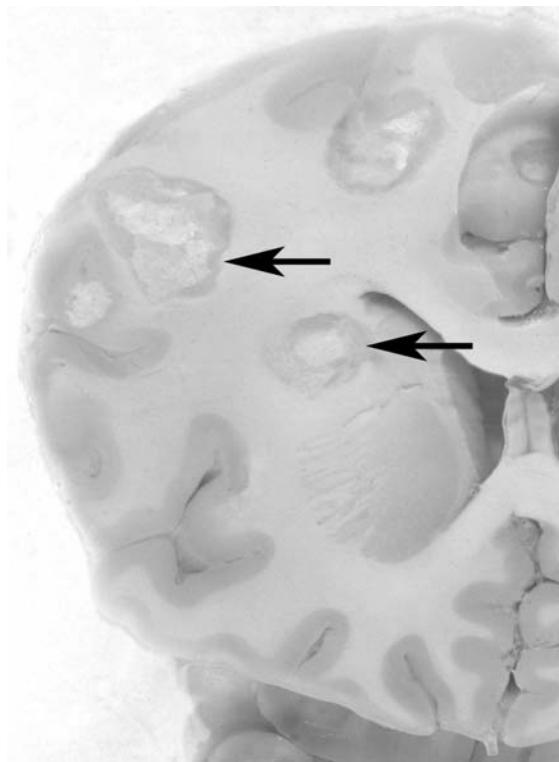


Fig. 32. Several cerebral abscesses (arrows) are seen in this coronal section of right frontal lobe. Note the ring of collagen around each abscess and how the abscesses tend to occur at the gray-white borders.

Most of these form at the gray/white junction because the caliber of cerebral arterioles diminishes at this location, and infected, blood-borne particles (*septic emboli*) get stuck within the narrowed lumen. (This same concept holds true for metastatic tumors). The brain tries to “wall off” the abscess with collagen, so abscesses show a thin, gray rim of collagen surrounding the white center of necrotic debris. It is important to note that this rim of collagen is not present around metastatic tumor nodules, which can otherwise resemble abscesses grossly.

Another type of space-occupying infectious lesion is known as a *granuloma*. These can be caused by tuberculosis, fungal infections, or by parasites. In the United States, parasitic brain infections are fairly rare, but trichinella parasites from incompletely cooked pork can encyst in the brain and cause granulomas.

Approximately 40% of brain abscesses are a secondary consequence to disease in the paranasal sinuses, the middle ear, or mastoid process air cells. Another 30% of brain infections arise from metastases of infections elsewhere, most frequently the lungs or the heart valves (*endocarditis*). Clinically, these patients present with headache that proceeds



Fig. 33. A contrast-enhanced CT scan displays a typical ring-enhancing lesion caused by an abscess. Note the very round appearance of the lesion and the dark center. (Compare with the irregular ring-enhancing lesion caused by a tumor shown in Fig. 30.) The bright ring represents a disrupted blood-brain barrier that has allowed contrast material to leak out of the capillaries. The dark center represents necrotic material (pus). Edema (dark signal) surrounds the abscess.

to drowsiness and confusion. There may be focal or generalized seizures. Fever is present until the infection becomes encapsulated.

4.1. Meningitis

In addition to space-occupying lesions, infections may also produce diffuse or infiltrative lesions such as *meningitis* (Fig. 34) and *encephalitis*. Meningitis is an infection of the meninges (usually bacterial) whereas encephalitis is an infection of the brain itself (usually viral). Bacteria can gain access to the CSF in the subarachnoid space by way of the blood, as in *septicemia*, or via metastases from infections elsewhere in the body. The most frequent causative agents (>80% of cases) are *Haemophilus influenzae*, *Neisseria meningitidis*, or *Streptococcus pneumoniae*. The overall mortality rate from *untreated* bacterial meningitis is 50% to 90%, but with the use of antibiotics it is now <10% with most dying within 48 h of hospital admission. The patient is acutely ill, and the symptoms include chills, fever, headache, nausea, vomiting, stiffness of the neck, and prostration.

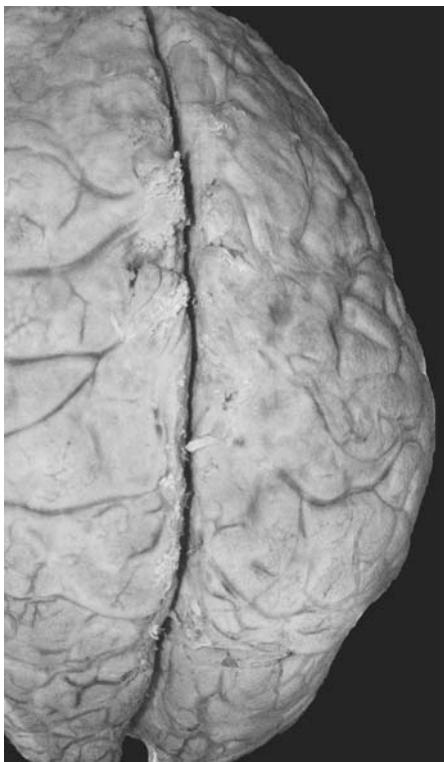


Fig. 34. This is an example of purulent meningitis: an infection of the meninges. The arachnoid of the dorsal aspect of the brain is shown. Note how a layer of pus has developed beneath the arachnoid membrane. The opacification of the arachnoid by a chalky infiltrate of polymorphonuclear leukocytes makes it impossible to discern the normal underlying gyral structure. Meningeal veins are easily discerned as the purulent meningitis has caused an engorgement of the blood vessels within the arachnoid.

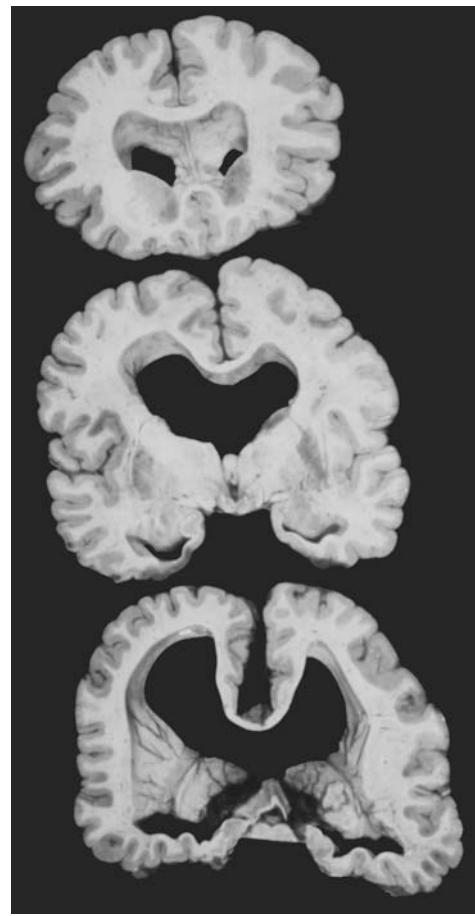


Fig. 35. Several coronal sections through a brain with marked enlargement of the ventricles (hydrocephalus).

5. PERINATAL ABNORMALITIES: HYDROCEPHALUS

Hydrocephalus means enlarged cerebral ventricles (Fig. 35). This is not a specific disorder in itself, but rather an end result of various disorders. Hydrocephalus may result from blockage of CSF flow at some point (the usual cause), from increased CSF production (rare), or from simple compensatory dilation due to loss of cerebral substance (cerebral atrophy). This latter category is of little clinical significance. If CSF is prevented from reaching the subarachnoid space, then the condition is called *noncommunicating (obstructive) hydrocephalus*. Blockages can occur at the level of the interventricular foramen, the cerebral aqueduct, or the lateral and median apertures. If the CSF resorption from the subarachnoid space (via *arachnoid granulations*) into the venous system is impeded, the condition is called a *communicating hydrocephalus*.

Congenital hydrocephalus is found in 0.5 to 1.8 per 1000 births. The most frequent cause of congenital hydrocephalus is a stenotic cerebral aqueduct resulting in a noncommunicating hydrocephalus. The lateral and third ventricles will be excessively large, whereas the fourth ventricle will be of normal size. In some cases, there may be a complete absence of the cerebral aqueduct. Because an infant's cranial sutures have not yet closed, the head is able to expand to accommodate the extra fluid (Fig. 36), and there is less actual damage to brain tissue. Other causes of congenital hydrocephalus include the *Dandy-Walker syndrome*, where the expansion of the fourth ventricle blocks the lateral and median apertures, and the *Arnold-Chiari malformation*, where the cerebellum protrudes through the foramen magnum. In these cases, the lateral and median apertures are compressed and closed.

In infants, as the CSF pressure increases, there are increasing problems with eye movements and pupillary reactions. An absence of upward gaze results in



Fig. 36. This infant has hydrocephalus due to stenosis of the cerebral aqueduct. Because the fontanels have not fused at this early age, the increased intracranial pressure causes the head to enlarge. Note that the eyes turn down (i.e., *setting sun sign*), so that the sclera is visible above the iris. This sign is caused by increased intracranial pressure that compromises the vertical gaze center in the dorsal midbrain.

eyes that are directed downward with the sclera visible above the irises (i.e., the *setting sun sign*) (Fig. 36). Untreated hydrocephalic infants show mental and motor retardation, progressive weakness, and seizures. The defect can be corrected by placing a shunt into a lateral ventricle that drains the CSF into the peritoneal cavity, where it is resorbed into the venous system. If left untreated, the mortality rate is 50% at 1 year of age and 75% by 10 years of age. In adults, the symptoms include headache, lethargy, malaise, and weakness.

Communicating hydrocephalus can result from postinflammatory infections of meningitis or tuberculosis. Subarachnoid bleeds can also block (clog) the arachnoid granulations and impair their function. Rarely, there is a congenital absence of arachnoid granulations.

6. CONGENITAL MALFORMATIONS

There are important neurodevelopmental stages that must occur for proper CNS growth and function. Clearly, the proper closure of the neural tube, to protect it from amniotic fluid, is essential. About 60% of these neural tube defects are of an unknown cause, and about 12% are due to environmental causes such as maternal infections, toxins, and malnutrition. Only 13% have been associated with chromosomal defects, and fewer still with single-gene abnormalities. There are a plethora of congenital abnormalities, and this section only examines the most prevalent and a few of the more unusual.

6.1. Neural Tube Defects

Defects in dorsal induction cause *neural tube defects* that occur prior to, or during, the formation of the neural tube. (Note: The defects discussed in this section have occurred during the process of primary neurulation, which forms the brain, brain stem, and spinal cord down to and including the S1 segment.) These defects occur during weeks 3 to 4 of gestation. Neural tube defects fall into several broad categories, but these categories blend with one another so that a gradient of deficits exists. For example, the entire neural groove may fail to fuse into a neural tube, with the resultant failure of brain, brain stem, and spinal cord development. This worse-case defect is known as *craniorachischisis totalis* (Fig. 37). Anterior neuropore defects predominate in females, whereas posterior neuropore defects predominate in males. The reason for this sex difference is not understood.

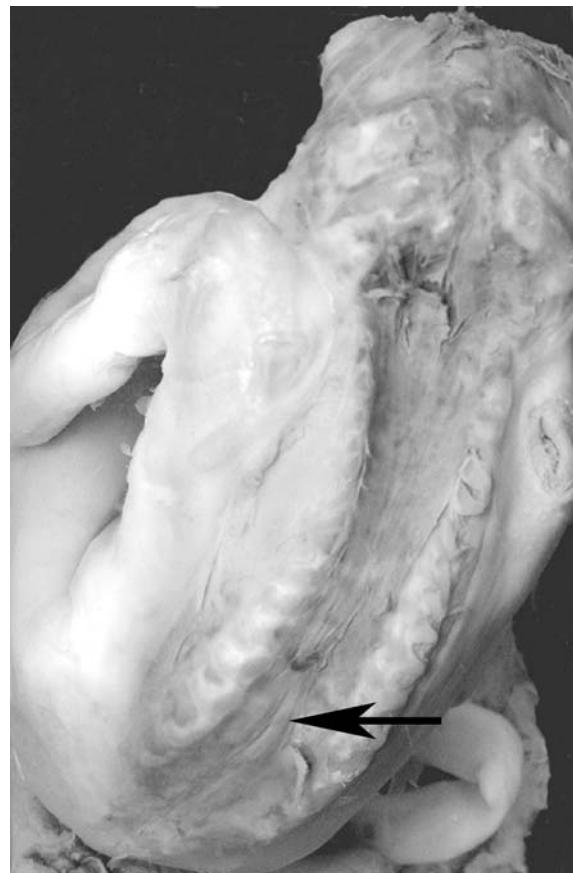


Fig. 37. A dorsal view of craniorachischisis totalis. Note the lack of brain and spinal cord. Because the neural tube never formed, the entire cranial vault and dorsal aspect of the vertebrae remained open to the *in utero* environment (i.e., amniotic fluid). The neural folds and groove can be seen in the caudal aspect of the vertebral column (arrow).

Exposure of the neural tube to amniotic fluid past the times when the anterior and posterior neuropores are to close is deleterious to neural development. Ultrasound examination of the fetus is now used to identify neural tube defects at an early stage. This can be combined with an amniotic fluid test to determine levels of α -fetoprotein, which normally drop markedly once the neural tube closes. If levels remain high, then the presence of a neural tube defect is likely. Over the past few decades, it has been found that supplementing the diet with *folate* for women who are planning to, or may, become pregnant can dramatically reduce the incidence of neural tube defects.

The least severe form of neural tube defect is *spina bifida occulta* where the vertebral arch (i.e., laminae and spinous process) of a single vertebra, usually in the low lumbar or upper sacral levels, fails to fuse along the midline.

6.1.1. ANENCEPHALY

Figure 38 shows anencephaly, a very common neural tube defect that results from the failure of closure of the *anterior neuropore* around gestational day 25. Depending on how much of the anterior neuropore has closed, there is either a complete or partial absence of the prosencephalon (i.e., telencephalon and diencephalon). In either case, the prosencephalon is represented by a disorganized, nonfunctional mass, and the cranium is deficient. Many of these infants are born alive but succumb within 1 to 4 days to massive infections resulting from the exposed hemorrhagic neural material. Because the brain stem (mesencephalon and



Fig. 38. A close-up posterior view of anencephaly. There is an absence of development of telencephalic structures and the flat bones of the calvaria due to failure of anterior neuropore closure. The diencephalon and more caudal neural structures would be present. Note how the neural tissue blends into the skin of the head as both are of ectodermal origin.

rhombencephalon) develops, a rudimentary sucking reflex is present, and heart rate and breathing centers are intact. The cerebellum, a derivative of the metencephalon, and the spinal cord are also present, although the spinal cord is consistently small due to failure of development of the corticospinal tracts. Anencephaly occurs in 0.1 to 0.7 per 1000 births with a female predominance of 3:1 to 7:1. In many cases, anencephaly is combined with other neural tube defects that may, or may not, have a direct physical connection to the cranial defect.

6.1.2. POSTERIOR NEUROPORE DEFECTS

Frequently, the *posterior neuropore* starts to close (this begins in the cervical region) but does not do so completely by gestational day 27. The brain, brain stem, and spinal cord to the level of the defect are normal. Posterior neuropore defects are named according to the extent of involvement of bone, meninges, and spinal cord in the malformation. In *spina bifida occulta* (not shown), the defect involves only the vertebral arch that fails to fuse along the midline; it is covered by skin and does not involve the spinal cord or the meninges. Usually, the external sign is a pigmented spot and/or a small tuft of hair on the midline of the lower back. Less frequently, a small blind-ended dimple is present, or a sinus (tract) will connect the external environment with the dural sac so that CSF can leak out and/or bacteria can enter into the subarachnoid space resulting in repeated episodes of meningitis. It is estimated that up to 10% of the population may have *spina bifida occulta*, with no neurologic or detrimental health consequences. *Spina bifida occulta* is seen regularly in cadavers used in our gross anatomy laboratory. *Spina bifida occulta* often occurs at the site of juncture between primary and secondary neurulation.

The next most severe form of posterior neuropore defect is a *meningocele*. Here there is a defect in the bony structure of the posterior arches, and in addition a dural sac and an enlarged subarachnoid space make a skin-covered bulge on the back, whereas the spinal cord is normal and lies in its normal position against the vertebral bodies. Yet more severe is a *meningomyelocele*, in which the bulging dural sac also contains an abnormally formed spinal cord (Fig. 39). The bulge is covered by a thin, semitranslucent, weeping membrane consisting of malformed meninges. Meningomyeloceles are 10 times more frequent than meningoceles.

The degree of spinal cord function depends on the presence and extent of spinal cord involvement in the malformation. If the lumbosacral spinal cord is



Fig. 39. An infant with a meningomyelocele due to failure of posterior neuropore closure. The large weeping defect contains vascular, disorganized neural tissue. Note that no skin covers the defect. There is a high probability that this infant has an Arnold-Chiari defect and is at risk of also developing hydrocephalus (see Fig. 40).

defective, there may be loss of bowel or bladder control, and the hips and legs may be atrophied and nonfunctional. Recent surgical and pharmaceutical advancements now permit many individuals with meningomyeloceles to live until adulthood. Because their brains are normal, these individuals can be productive in the workforce. However, shortly after birth, individuals with meningomyeloceles must be watched carefully for the development of hydrocephalus (see below).

6.1.3. A CORRELATION EXISTS BETWEEN MENINGOMYELOCELES AND THE DEVELOPMENT OF HYDROCEPHALUS

Figure 40 shows an infant with a large lumbosacral meningomyelocele and advanced hydrocephalus. (This infant was born in the 1940 s, before successful surgical procedures were developed that could shunt the constant CSF production into the peritoneal cavity for absorption.) These two disorders—meningomyelocele and hydrocephalus—can often be found together. Almost always associated with this combination of defects is a *Arnold-Chiari malformation* where contents of the posterior cranial fossa (cerebellar vermis and/or cerebellar tonsils and medulla) are found tightly packed into the upper vertebral canal. One theory is that it is a physical process that causes the vermis and medulla to be pulled into the vertebral canal. Because a meningomyelocele causes the spinal cord to be tethered to the vertebral column, the spinal cord cannot move in relation to the vertebrae as they grow during development; consequently, a portion of the cerebellum and



Fig. 40. An infant with a large lumbosacral meningomyelocele (arrow) and advanced hydrocephalus. These two disorders—meningomyelocele and hydrocephalus—are often found together and are associated with an Arnold-Chiari malformation where contents of the posterior cranial fossa (cerebellar vermis and/or cerebellar tonsils and medulla) are found in the upper vertebral canal.

medulla are pulled through the foramen magnum. This herniation impedes the flow of CSF through the lateral and median apertures into the subarachnoid space and causes a noncommunicating hydrocephalus. However, it is important to note that there are many individuals with meningomyeloceles that do not develop hydrocephalus.

6.2. Encephaloceles

Figure 41 shows an infant with an *encephalocele*. An encephalocele occurs when there is a mesenchymal defect/deficiency that occurs during the development of the squamous portion of the occipital or frontal bones. More than 80% to 90% of encephaloceles occur on the occipital region. The resultant opening permits a portion of the meninges and/or CNS to protrude through the bony defect. Bony defects in the frontal bones also occur but are much rarer. Depending on what protrudes through the bony defect—meninges, or meninges plus CNS tissue, or meninges, CNS tissue, and a part of the ventricular system—



Fig. 41. This live-born infant has a “small” encephalocele due to a defect in the occipital bone. Because we cannot see beneath the skin, it is unknown if just the meninges are involved or if a portion of the CNS and the ventricles are also herniated through the bony defect. The most benign situation would be the presence of a meningocele.

encephaloceles have different names: *meningocele*, *meningoencephalocele*, or *meningohydroencephalocele*, respectively. Therefore, the term *encephalocele* is generic and is used when the physician does not yet know what lies beneath the skin- or meninx-covered herniation. In large meningohydroencephaloceles, the occipital lobes, the cerebellum, and greatly enlarged occipital horns of the lateral ventricles may protrude through the bony defect. In extreme cases, an encephalocele may be as large as the head of the infant.

Recent surgical advancements have provided an opportunity for surgeons to operate *in utero*. When an encephalocele is discovered, usually via ultrasound, a cesarean section is performed, the head of the infant is pulled out of the uterus, and the defect is repaired as best as possible. The fetus is replaced in the uterus and allowed to develop. Such surgical techniques increase the possibility of reducing the severity of CNS damage.

6.3. Malformations Caused During Gastrulation

Figure 42 shows an infant born with dicephaly. In brief, during the process of *gastrulation*, the *notochord* induces the development of the *primitive streak*. In turn, the primitive streak induces the overlying epiblast cells to differentiate into the neural plate, which rolls up and closes to form the neural tube from which the brain and spinal cord develop. In this instance, the rostral aspect of the primitive streak was duplicated, which resulted in the duplication of

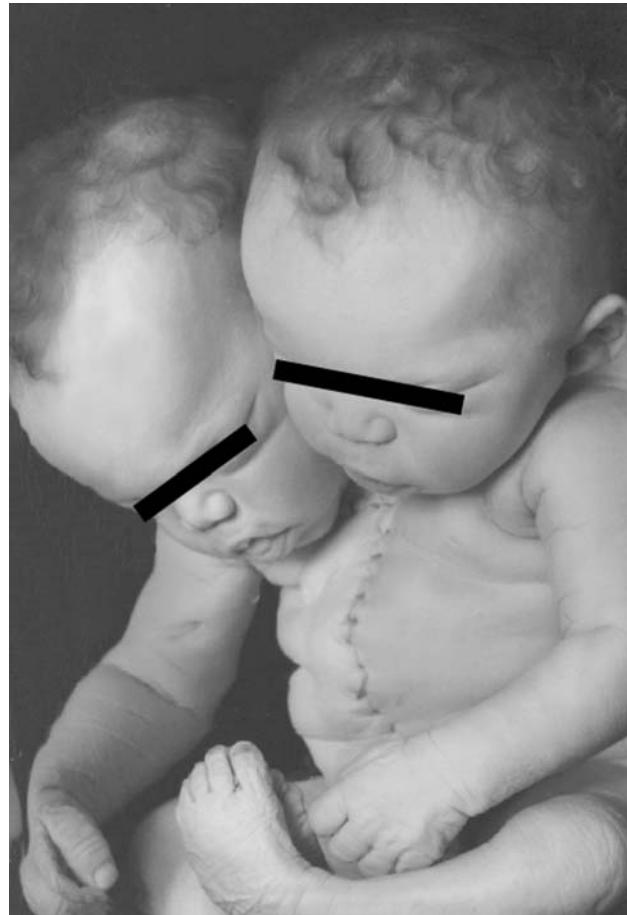


Fig. 42. Dicephaly (more precisely, *dicephalus dipus dibrachius*: a fetus with two heads but only two feet and two arms). An unknown insult during early gastrulation caused a duplication of the rostral portion of the neural tube. Duplication of the neural tube went on to form two heads. The thorax was opened (*stitches*) during the autopsy to examine the internal organs.

the rostral aspect of the neural tube. A malfunction of the homeobox gene *Lim-1* is implicated, as *Lim-1* is known to help organize the primitive streak. The overexpression of the transcription factor *Goosecoid* may also be involved, as injections of *Goosecoid* mRNA into tadpoles induces the formation of duplicate heads.

Other defects in gastrulation result in a deficient caudal cell mass that is essential for secondary neurulation to occur. This defect is termed *caudal regression syndrome* (also called *caudal dysgenesis*). This defect can be either mild or severe. In more mild forms, there are hypoplastic femurs and sacrum. There would also be a malformed S2 through C01 spinal cord segments. Normal limb and bone development depends upon normal innervation during development. In the most severe

form of caudal regression syndrome, the legs are fused together with no feet (*sirenomelia*), the external genitalia are missing, and the pelvic organs would be malformed. Less severe forms are characterized by varying degrees of lower extremity malformations, anomalies of lumbar, sacral, and coccygeal vertebrae, imperforate anus, agenesis of the kidneys and urinary tract, and agenesis of the internal reproductive organs, with the exception of the gonads.

6.4. Ventral Induction Defects

These defects occur on the ventral and rostral aspects of the embryo that are controlled by prechordal mesoderm. The most severe ventral induction defect is *holoprosencephaly* (Fig. 43 and Fig. 44). Because the prosencephalon fails to evaginate from either side of the neural tube, the telencephalon and diencephalon are not paired. Because the retinas are an extension of the diencephalon, there is either a single midline eye (*cyclopia*) or, in the case shown (Fig. 43), fused eyes (*synophthalmia*). A single nasal cavity with a misplaced nostril/proboscis (*cebocephaly*) is present. The neural tube, caudal to the diencephalon, undergoes normal

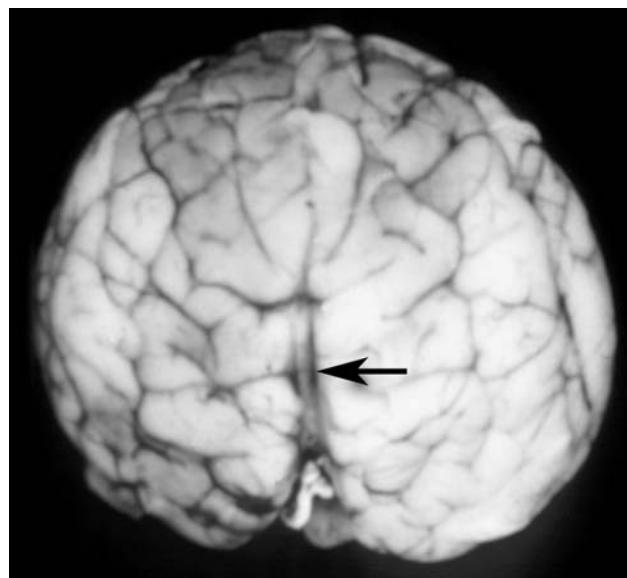


Fig. 44. A brain that shows almost complete holoprosencephaly. The telencephalic vesicles started to bulge outward from the rostral neural tube, as revealed by the beginning of a longitudinal sulcus on the ventral surface of the frontal lobe (arrow), but never completed the process.



Fig. 43. An infant with holoprosencephaly. Note the almost complete fusion of the two eyes and the misplaced proboscis. Internally, there would be one fused cerebral cortex, a single midline diencephalon, and no corpus callosum (see Fig. 44). The lateral and third ventricles would form one large central cavity.

development; therefore, the brain stem and spinal cord undergo normal formation.

Holoprosencephaly presents as a spectrum of deficiencies, depending on when this defect started to develop. For example, if the prosencephalon started to evaginate, but never completed the process, then the degree of severity of holoprosencephaly would be reduced. In the least severe form, the holoprosencephalic malformation may be as inconspicuous as the absence of the olfactory bulbs. In milder forms, the infant survives and displays varying degrees of mental retardation, motor abnormalities, spasticity, and an inability to regulate body temperature. The corticospinal tracts and corpus callosum are always missing in holoprosencephaly as these structures are also under the control of ventral induction factors.

6.5. Disorders of Migration

Figure 45 shows an example of *polymicrogyria*. In this condition, the “true” gyri are subdivided into a number of fused miniature pseudogyrus. This condition is thought to result from a primary defect in the migration of neurons once they have formed from the proliferative layer of the neural tube. It may also be due to an inadequate formation of neurons and glia or from some type of anoxic-ischemic event, as polymicrogyria can be confined to a single vascular territory. Infection with *cytomegalovirus* may also be

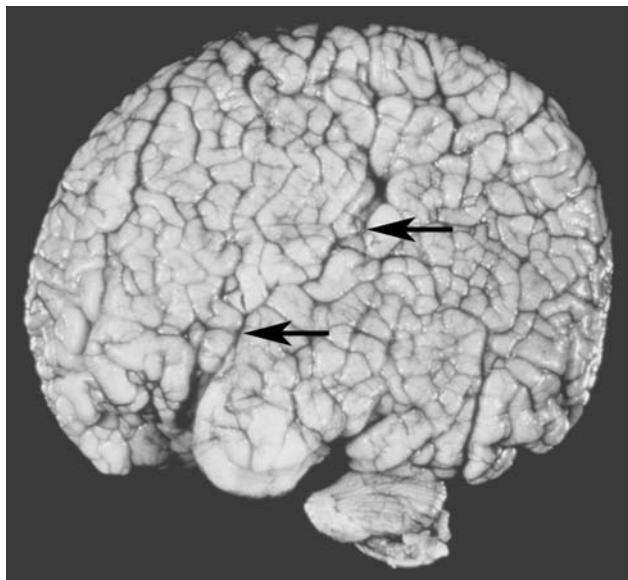


Fig. 45. Polymicrogyria over the entire cerebral cortex. Note the impression of numerous additional miniature gyri. The only anatomic landmark that is evident on this brain that enables a distinction of one lobe from another is the lateral sulcus (*arrow*).

causative. Polymicrogyria can be unilateral, bilateral, diffuse, or occur in small patches. The example shown is a global polymicrogyria. Some of these migratory defects are the result of certain genes, especially the *Hox* family, not functioning properly during development.

There are several other types of migratory defects. *Agyria* (*lissencephaly*) is characterized by a smooth brain with very few gyri. It is thought that the proliferative zone of the prosencephalon fails to produce adequate numbers of neurons and glia. Those that are produced are stacked on top of each other from the ventricular system to the pial surface, forming, in essence, one large mantel layer that has no recognizable white-matter core that is characteristic of the telencephalon. *Pachygyria* is a less severe form of agyria. These brains have a greatly diminished number of broad gyri. In both cases, the brain is smaller than normal, and the lateral ventricles are slightly enlarged.

Heterotopias are islands of neurons that are misplaced in the CNS. Heterotopias, by definition, have to occur in the white matter. They are often found surrounding the ventricular system and represent clusters of neurons that never migrated away from the embryonic proliferative layer of the neural tube. Heterotopias often develop into a focal point for seizure activity.

Brain size is generally not a factor in determining intelligence, unless the weight is significantly outside the normal range of 1200 to 1600 g. One might assume that a very large brain would confer great intelligence, but most excessively large brains are associated with mental retardation, as are most small brains.

6.6. Schizencephaly/Porencephaly/Hydranencephaly

These conditions result from destructive defects late in gestation. These lesions are not really malformations because the brain and skull are first normally formed before portions are lost. These lesions are thought to result from vascular occlusions after neurogenesis has occurred. The loss of blood supply causes tissue necrosis, leaving either clefts (*schizencephaly*) or holes (*porencephaly*) in the brain (Fig. 46). In the most extreme case, all or most of the cerebrum is lost, leaving a normal-appearing but CSF-filled head (*hydranencephaly*). Some cases of schizencephaly have been linked to a gene known as *EMX2*.

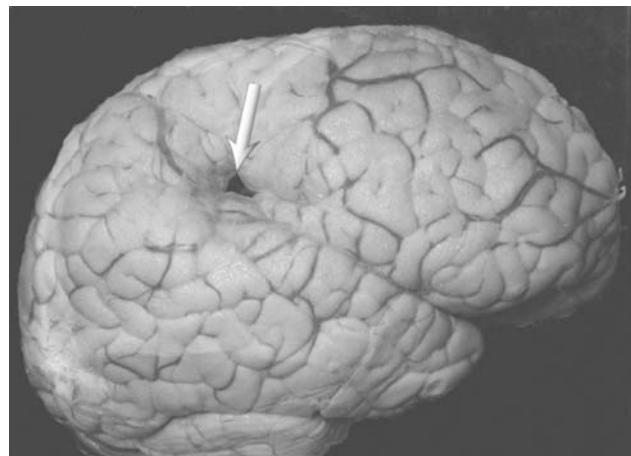


Fig. 46. Porencephaly of the right cerebral cortex. Note the small hole in the cerebral cortex (*arrow*) just dorsal to the lateral fissure where the parietal, temporal, and occipital lobes come together. This defect is open to the underlying ventricular system.

7. OTHER COMMON PATHOLOGIES

There are numerous other types of neural defects. This section mentions several more common types of neurologic problems physicians will encounter. Some occur in adulthood, whereas others are developmental in nature.

7.1. Cortical Atrophy

Figure 47 shows a brain that has pronounced atrophy of the cerebral cortex. Compare this brain with one that does not display atrophy (see Fig. 14 in Chapter 2). Note that the sulci are wide in the atrophied brain due to death of neurons that cause shrinking of the gyri. Some degree of atrophy occurs with normal aging, and brain weight decreases by 11% in many individuals from the ages of 18 to 85.

Atrophy is more pronounced in a number of diseases that cause neuron loss and dementia. The most common of these is *Alzheimer's disease*, where cortical atrophy occurs in the association areas of the cortex and in the hippocampus. More than 35% of pyramidal neurons are lost in Alzheimer's disease, and there is an 80% loss of cholinergic neurons in the *nucleus basalis*, with the cortex showing a concomitant reduction in acetylcholine-containing fibers. In this progressive disease, the patient slowly withdraws from life as they eventually become unable to perform simple tasks or remember their closest friends or spouse. In essence, the patient displays a reverse developmental profile. Earlier onset of disease is associated with more rapid progression of cortical deterioration. The end stages of Alzheimer's disease is characterized by complete loss of higher mental functions.

There are other types of cortical atrophy and some predominantly affect particular regions; for example,

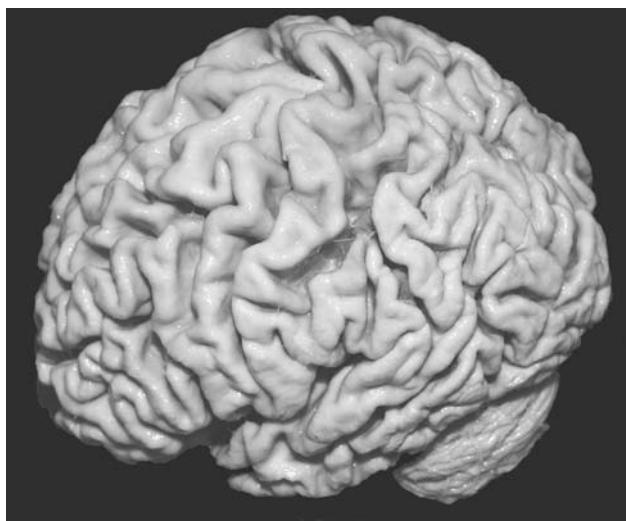


Fig. 47. The lateral surface of an adult brain that has atrophied. Note the wide sulci and thin gyri. The atrophy is particularly pronounced in the frontal and parietal lobes (i.e., regions of the cortex that contain vast amounts of association cortex). Compare this brain with normal brains seen in Chapter 2.

in *Pick's disease*, atrophy is most marked in the frontal lobe and parts of the temporal lobe. Another cause of dementia is *prion* diseases (e.g., *Creutzfeldt-Jakob disease*). Prion diseases have an insidious onset, and the gestational period may be as long as decades. They can be acquired by ingesting infected nervous tissue, but the vast majority of cases of human prion disease appear to result from spontaneous conversion of normal human proteins into pathogenic prions.

7.2. Multiple Sclerosis

Figure 48 and Fig. 49 show brains from individuals with *multiple sclerosis* (MS). MS is an autoimmune disease in which the immune system attacks and destroys myelin produced by oligodendrocytes. This shows up grossly as foci (plaques) of discoloration in the white matter. During an “attack,” there is active destruction of myelin in the plaque, but then the inflammation subsides and a focus of myelin loss remains. Repeated attacks leave numerous plaques randomly distributed through the brain and spinal cord. The disease usually starts in early or mid-adulthood and is characterized by repeated attacks that occur at different times over many years. Each attack brings new clinical symptoms that vary widely and can be very confusing before the correct diagnosis is made. The disease itself is rarely fatal, but patients endure repeated and increasing problems, often over the course of decades.

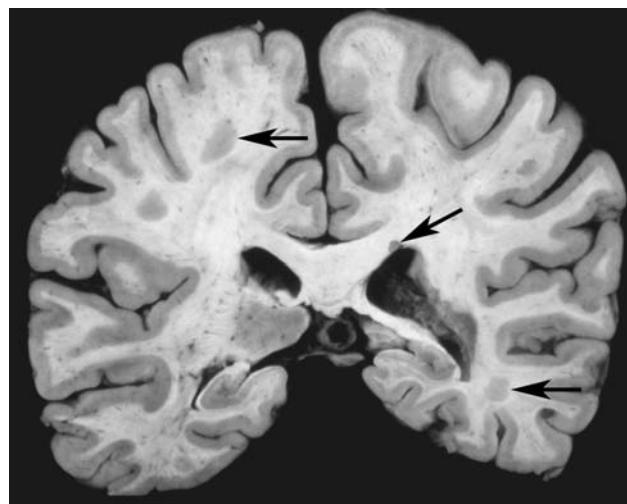


Fig. 48. A coronal section through a brain that contains multiple sclerosis plaques (arrows). These plaques are generally located in the white matter as this disease destroys myelin. However, there are myelinated axons in gray areas of the brain as well, and plaques can also occur there.

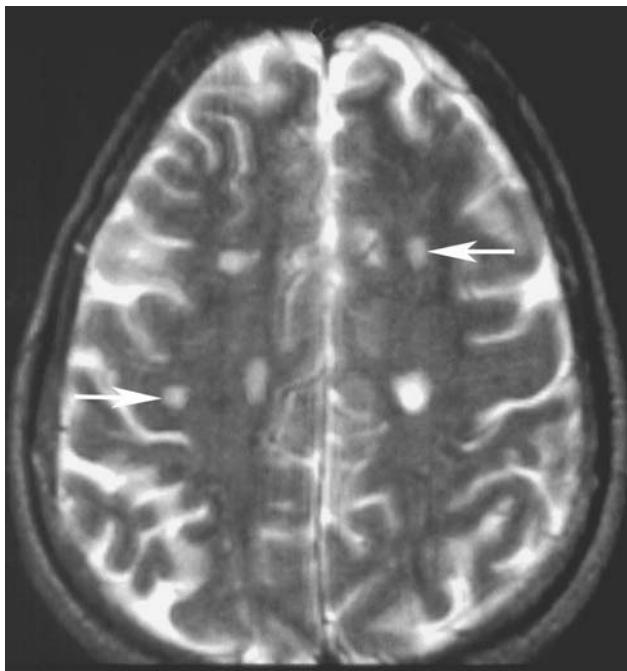


Fig. 49. A T2-weighted MRI scan of a brain of a patient with multiple sclerosis. The plaques (arrows) appear white on MRI and have a predilection to form around the ventricular system. This horizontal plane of section is just dorsal to the lateral ventricles.

7.3. Huntington's Disease

Huntington's disease is an autosomal dominant genetic disorder resulting from numerous (>37) CAG repeats on chromosome 4. Normal individuals have 11 to 34 CAG repeats. The more CAG repeats present (and some people have >80), the more “aggressive” the disease is. The incidence rate is 5 to 7 cases per 100,000 people. Individuals with Huntington's disease have a degeneration of the caudate and putamen (striatum) of the basal ganglia with marked gliosis. Up to 95% of striatal neurons may degenerate. Huntington patients usually start to show symptoms between 35 and 40 years of age when *choreiform* and *athetotic* movements of the limbs and face become pronounced. Thus, in the past, many people with Huntington's disease already reproduced before they knew they had the disease. Now, with genetic screening, a person may know they carry the gene and choose not to pass the gene on to their children. In later stages of the disease, patients may develop a parkinsonian-like rigidity. Because the striatum has massive reciprocal connections with the cerebral cortex, as the disease progresses the individuals become depressed and demented. There is, as yet, no cure for this disease.

7.4. Parkinson's Disease

Another common neurologic pathology is *Parkinson's disease*. There is an incidence rate of 8 to 18 cases per 100,000 population. The onset of Parkinson's disease is insidious and is often unilateral at first before becoming generalized. The disease is caused by a progressive loss of dopamine-producing neurons in the substantia nigra and does not become evident until the loss is $>80\%$. As the disease progresses, there is also a loss of 50% to 80% of the serotonergic and noradrenaline-producing neurons in the raphe and locus coeruleus, respectively. Up to 50% of the acetylcholine-producing cells of the nucleus basalis may also degenerate. There is a familial component to Parkinson's disease and there are undoubtedly environmental factors involved. Curiously, Parkinson's disease was not described until 1817, after the advent of the industrial and chemical revolution.

The symptoms of individuals with Parkinson's disease include a tremor at rest, bradykinesia (slowed movement), hypokinesia (decreased amount of movement), a shuffling walk, loss of balance, and a loss of emotional facial expressions (*masked facies*). In advanced cases, there is rigidity of the muscles. Speech is rapid, monotone, and soft. Replacement of the lost dopamine with *L-DOPA* (*L-DOPA* crosses the blood-brain barrier, whereas dopamine cannot) helps ameliorate the symptoms but eventually becomes ineffective. Stereotaxic lesions in the internal globus pallidus can reduce or eliminate tremor and rigidity for a period of time. More recently, deep brain stimulation of the globus pallidus with implanted electrodes can be used to disrupt the neural signals contributing to tremor and rigidity.

7.5. Disorders of Organization

Organizational defects can occur when the axons of a particular population of neurons fail to reach their proper targets or if they form synapses on the wrong part of the target neuron. The developmental organization of synaptic contacts is precisely controlled: down to the level of the number of synapses formed on a neuron and its cytoplasmic extensions, as well as the location of synapses with respect to the neurotransmitter(s) they contain.

Organizational disorders can result from an inadequate number of synapses being formed. Maternal malnutrition, alcohol consumption, and drug abuse are the most common causes of inadequate synapse formation. *Congenital rubella*, which is now controlled by vaccination, used to be a contributing factor. *Mental retardation* can be a result of an inadequate

number of synapses. (However, it must be mentioned that nearly 40% of mentally retarded individuals show no gross or microscopic CNS alterations.) Mental retardation is by far the largest neurodevelopmental disorder in the world and is estimated to affect 2.5% to 3% of the population, with males outnumbering females 3:1. Mental retardation can be divided into several categories depending on the severity: profound ($\text{IQ} < 25$), severe ($\text{IQ} = 25 \text{ to } 39$), moderate ($\text{IQ} = 40 \text{ to } 54$), and mild ($\text{IQ} = 55 \text{ to } 69$). There are about 600,000 severely retarded individuals in the United States that require constant care in specialized institutions. There are no treatments to cure retardation as there is no way to undo the developmental disorganization. Thus, the objective of the physician and family members is to assist in training and making vocational education and social adjustments. Destructive lesions account for 26% of severely retarded individuals, and chromosomal abnormalities account for 18% of severely or mildly retarded persons. Down's syndrome

(trisomy 21) is a major contributor to mild retardation. Low-grade lead poisoning or mercury poisoning are also causes.

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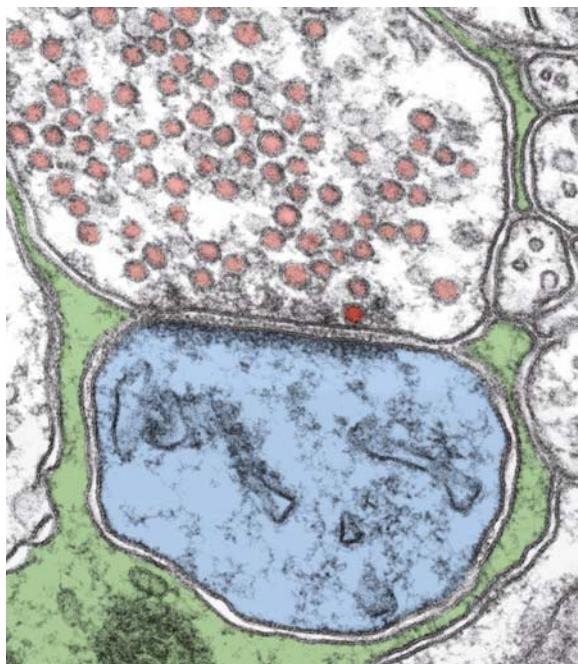
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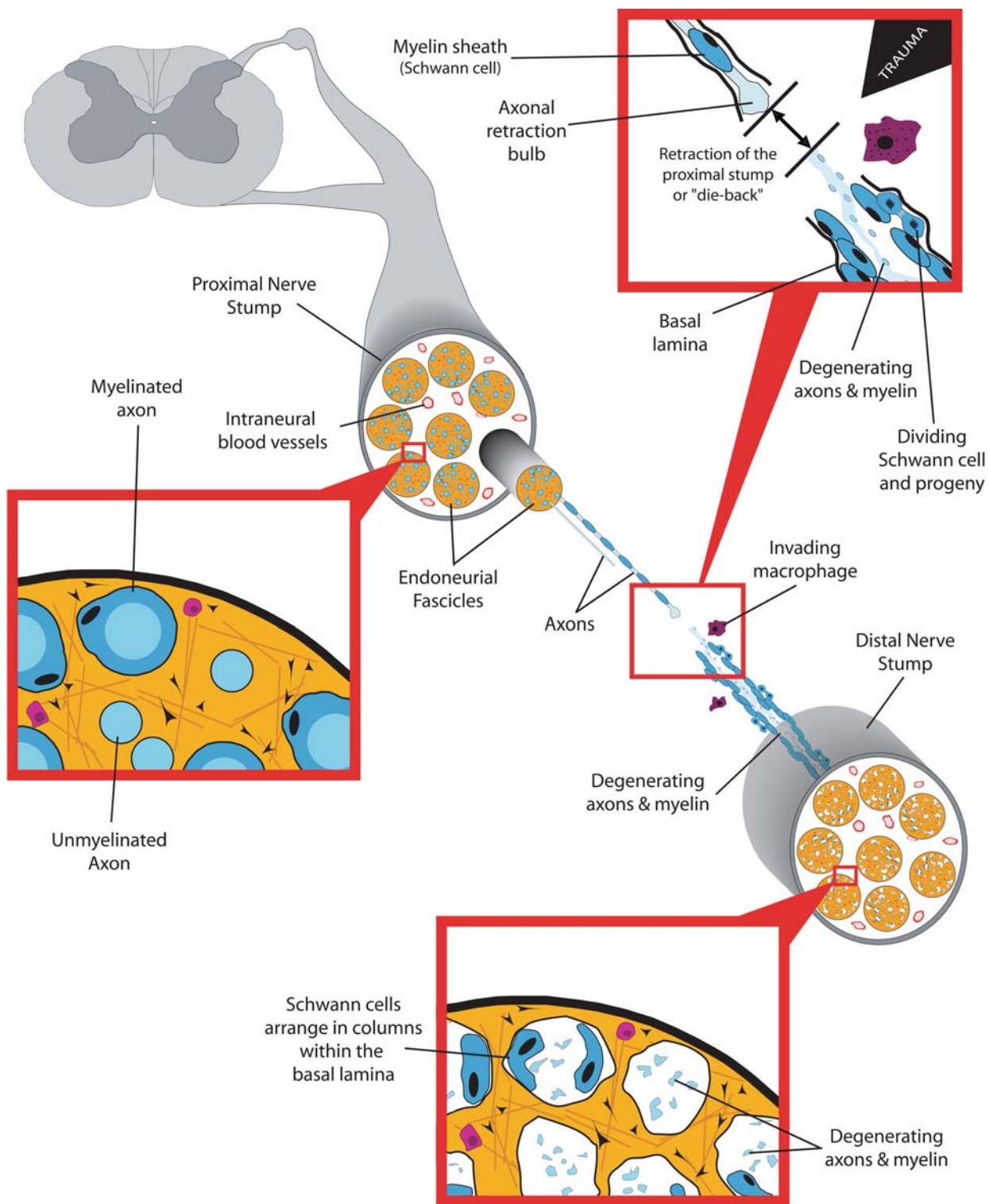
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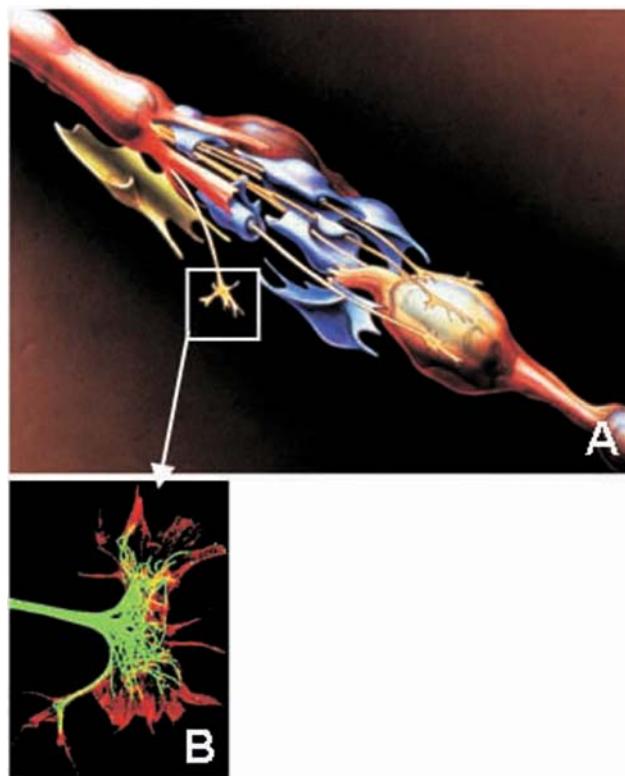
COLOR PLATES



Color Plate 1. An electron micrograph of a CNS synapse. This example of a synaptic bouton-type synapse is located in the “molecular layer” of rat cerebellum. A single *en passant* bouton of the parallel fibers synapses onto a single Purkinje cell spine. Note the multiple synaptic vesicles in the presynaptic bouton terminal. Several vesicles seem to be linked by thin filaments in the cytoplasm. On average, the vesicles have a diameter of about 40 nm. One synaptic vesicle is clearly docked to the presynaptic membrane. Note also the narrow synaptic cleft, which contains a “fuzzy” set of electron-dense material (this probably includes cell adhesion proteins that span the cleft). The opposing postsynaptic membrane in the postsynaptic spine has an electron-dense postsynaptic density (PSD), where glutamate receptors and modulatory proteins are located. A thin glial process wraps itself around the synaptic cleft and postsynaptic spine and also partially around the presynaptic bouton-type terminal. (Electron micrograph courtesy of Constantino Sotelo, Instituto de Neurociencias de Alicante, Spain) (Chapter 4, Fig. 2; see discussion on p. 96).



Color Plate 2. A schematic diagram showing the sequence of events that leads to Wallerian (anterograde) degeneration. The normal cytology of a peripheral nerve is shown as a point of reference (expanded inset A). After axonal injury, the proximal stumps retract from the site of injury forming distinctive “retraction bulbs” (expanded inset B). Meanwhile, the distal portion of injured axons degenerate, but all other elements of the peripheral nerve remain intact (expanded inset C). Thereafter, Schwann cells begin to proliferate, and blood-borne macrophages infiltrate the degenerating nerve stump and assist Schwann cells with phagocytosis of axonal and myelin debris (expanded inset B). Schwann cells then become arranged in columns known as the “bands of Bungar” within common basal laminae (expanded inset C). Such Schwann cell units provide a cellular pathway along which regenerating axons extend distal to the site of injury (see also Fig. 10) (Chapter 31, Fig. 1; see discussion on p. 692).



Color Plate 3. (A) A three-dimensional rendition of early axonal regeneration after peripheral-nerve damage. Growth cones (e.g., *boxed profile*) are seen extending into the lesion gap (*blue profiles* representing connective tissue elements), and some make contact with Schwann cells (*red profiles*) in the distal stump. (Drawing kindly provided by Dr. Susan E. Mackinnon, Washington University School of Medicine and Barnes-Jewish Hospital.) (B) An axonal growth cone of an embryonic chick sensory neuron is shown. The growth cone is doubly stained with an antibody against tubulin (*green*), which labels microtubules, and rhodamine-phalloidin to label actin filaments *red*. The bundle of axonal microtubules (*green*) splays apart in the growth cone, and individual microtubules extend forward to interact with actin filament bundles and networks. These interactions between actin filaments and microtubules are important in determining directions of axonal growth and branching (*see text*). A small axonal sprout has formed at the lower left margin of the growth cone. (Figure generously provided by Paul C. Letourneau, Ph.D., University of Minnesota.) (Chapter 31, Fig. 9; *see discussion on p. 700*).