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THE EMERGENCE OF MODERN NEUROSCIENCE: Some Implications for Neurology and Psychiatry

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■ **Abstract** One of the most significant developments in biology in the past half century was the emergence, in the late 1950s and early 1960s, of neuroscience as a distinct discipline. We review here factors that led to the convergence into a common discipline of the traditional fields of neurophysiology, neuroanatomy, neurochemistry, and behavior, and we emphasize the seminal roles played by David McKenzie Rioch, Francis O Schmitt, and especially Stephen W Kuffler in creating neuroscience as we now know it. The application of the techniques of molecular and cellular biology to the study of the nervous system has greatly accelerated our understanding of the mechanisms involved in neuronal signaling, neural development, and the function of the major sensory and motor systems of the brain. The elucidation of the underlying causes of most neurological and psychiatric disorders has proved to be more difficult; but striking progress is now being made in determining the genetic basis of such disorders as Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, and a number of ion channel and mitochondrial disorders, and a significant start has been made in identifying genetic factors in the etiology of such disorders as manic depressive illness and schizophrenia. These developments presage the emergence in the coming decades of a new nosology, certainly in neurology and perhaps also in psychiatry, based not on symptomatology but on the dysfunction of specific genes, molecules, neuronal organelles and particular neural systems.

INTRODUCTION

One of the most remarkable developments in Biology in the last two decades has been the unprecedented growth of the traditional fields of Neurophysiology, Neuroanatomy, Neurochemistry, and Physiological Psychology, and concurrently, the gradual emergence of the new inter-disciplinary approach to the study of the nervous system which has come

> to be known as Neuroscience. Future historians of science will no doubt identify several elements that collectively contributed to this development, but even at this distance four factors can be recognized.
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> The first, and perhaps most significant, contributory factor has been the growing appreciation by both scientists and those who support their work, that few things are more important than understanding how the nervous system controls behavior. And with this, there is a growing sense that the study of the nervous system represents one of the great intellectual challenges of our time. By common consent Neuroscience has acquired much of the intellectual prestige and excitement that Molecular Biology had in the 1950s and 1960s, and it promises at least as much in terms of solid scientific achievement.
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> *Annual Review of Neuroscience*, 1978

With this prediction in its preface, the first volume of the *Annual Review of Neuroscience* was launched in 1978. The two subsequent decades have largely sustained this view. The continued success of neuroscience and its current excitement derive in large part from the incorporation into one intellectual framework of several previously independent disciplines. This synthesis has been gradual and can be seen in retrospect to have involved three overlapping steps. First, beginning in the 1950s and extending through the 1960s, there was the gradual merger into one unified field of neuroscience of several previously independent disciplines—neuroanatomy, neurophysiology, neuropharmacology, neurochemistry, and behavior. Second, the emergence of a coherent neuroscience was followed in the early 1980s by the integration of neuroscience with other areas of biology, particularly with molecular biology and molecular genetics, a direction that proved to have an immediate and profound influence on neurology and that may soon have an impact on psychiatry as well. The third step in this unification, which can be traced to the mid-1980s, was the merger of neuroscience with cognitive psychology. This merger has led to the formation of cognitive neuroscience, a coherent and systematic brain-based approach to mental function that promises in the long run to allow us to understand in a new way higher mental functions, perhaps even including selective attention and aspects of consciousness.

THE ORIGINS OF MODERN NEUROSCIENCE

The roots of neuroscience can readily be traced back to the latter part of the nineteenth century and to the first decade or two of the twentieth century. During this era, the structure and function of the nervous system first began to be studied rigorously by such towering figures as the anatomist Ramon y Cajal; the physiologists Sherrington, Langley, and Adrian; the neurologists Charcot and Hughlings Jackson; and the developmental neurobiologist Harrison. It is considerably

more difficult, however, to put a precise date on when "modern neuroscience" began. Cellular physiologists and biophysicists might cite, with justification, the publication of the seminal papers on the ionic basis of the nerve impulse by Cole & Curtis (1939) in the United States and by Hodgkin & Huxley (1939, 1952a–d) in England and the near contemporaneous work on the ionic basis of synaptic transmission at the neuromuscular junction by Fatt & Katz (1952). Much of this was brilliantly summarized and expanded to include work on the spinal cord, by Eccles in his Waynflete Lectures later published as the *Neurophysiological Basis of Mind* (Eccles 1953). Others of a more morphological persuasion might prefer a slightly later date, when the first informative electron micrographs of synapses in the brain and at the neuromuscular junction were published (Palade & Palay 1954, DeRobertis & Bennett 1954), and when Nauta's original method for tracing connections was introduced (Nauta & Gygax 1954). Still others, approaching the question from a molecular or developmental perspective, might date it to the discovery of the nerve growth factor by Cohen et al (1954) or to Sperry's (1963) formulation of the chemoaffinity hypothesis for the formation of specific patterns of connections. Still others would cite the work on the isolation and characterization of the subunits of the nicotinic acetylcholine receptor and the dramatic demonstration that the generation of antibodies against the receptor is the molecular basis of myasthenia gravis (Patrick & Lindstrom 1973).

Important though these landmark discoveries were, each was a contribution to one or another of the long-established disciplines, such as neurophysiology or neuroanatomy. None heralded what was to be the distinguishing feature of neuroscience as we now know it, namely its transcendence of traditional disciplinary boundaries. For this we have to turn to the first attempts to approach the problems posed by the nervous system in a unified, multidisciplinary way. At its most successful, this has been driven by problems rather than techniques, by an approach that has extended, in its reach, from the basic sciences of physiology, anatomy, and biochemistry to the biological foundations of behavior and to the cognate clinical disciplines of neurology and psychiatry.

David McKenzie Rioch and the Walter Reed Army Institute of Research

The initial attempt to transcend the conventional boundaries occurred in the mid-1950s, when David McKenzie Rioch, a psychiatrist well trained in neuroanatomy, brought together at the Walter Reed Army Institute of Research two remarkable groups of scientists: one consisting of scientists working on behavior and the other of scientists working on the brain. The behavioral group included the neuroendocrinologist John Mason, the experimental psychiatrists David Hamburg and Morton Reiser, and the behavioral psychologists Murray Sidman and John Brady. The brain group included Walle Nauta, the great Dutch neuroanatomist who developed modern methods for tracing connections in the brain; Robert Galambos, one of the leading physiologists of the auditory system; and Michael

Fuortes, at the time a promising Italian neurophysiologist. In addition to these more established people, Rioch also brought to his division a gifted group of postdoctoral fellows, including David Hubel, Ed Perl, Victor Wilson, and Felix Strumwasser. In this way Rioch succeeded in bridging psychiatric research on behavior—especially research on stress and depression—with basic anatomical and physiological studies of the nervous system.

FO Schmitt and the Neuroscience Research Program

The efforts of Rioch at the Division of Psychiatry at the Walter Reed were followed in 1962 by the establishment of the Neuroscience Research Program (NRP) at MIT by Frank Schmitt, who claimed to have coined the term neuroscience. The NRP was an interdisciplinary, interuniversity program made up of scientists from all areas of neuroscience as well as from chemistry, physics, immunology, genetics, and molecular biology, areas that Schmitt foresaw as ultimately having an impact on the brain sciences. The NRP sponsored stated meetings, workshops, and summer programs for senior and junior scholars. It published timely summaries of these deliberations in the form of the *Neuroscience Research Program Bulletins* and four large volumes based on the summer study programs it sponsored (Quarton et al 1967; Schmitt 1970; Schmitt & Worden 1974, 1979), which together helped to focus neuroscience on key problem areas of general scientific interest and thereby helped to bridge neuroscience to other areas of the biological sciences.

In an early program report in 1963, Schmitt summarized his views on the NRP.

> This 'new synthesis' [is] an approach to understanding the mechanisms and phenomena of the human mind that applies and adapts the revolutionary advances in molecular biology achieved during the postwar period. The breakthrough to precise knowledge in molecular genetics and immunology—'breaking the molecular code'—resulted from the productive interaction of physical and chemical sciences with the life sciences. It now seems possible to achieve similar revolutionary advances in understanding the human mind. A wealth of research literature on the mind stems from the classical approaches of physiology and behavioral sciences. By making full use of these approaches and by coupling them with the conceptual and technical strengths of physics, chemistry, and molecular biology, great advances are foreseeable. (quoted in Swazey et al 1975)

Stephen Kuffler and the Formation, At Harvard, of the First Neurobiology Department in the United States

These trends toward unification received their most coherent and forceful expression under the leadership of Steve Kuffler with the creation in 1967 of the first Department of Neurobiology in the United States at the Harvard Medical School.

As early as 1952, Kuffler had assumed directorship of the neurophysiology laboratory at the Wilmer Eye Institute at Johns Hopkins, where he began to gather a small group of gifted scientists interested in various areas of neurobiology. In 1959, he was invited to join the Department of Pharmacology at the Harvard Medical School to head the newly formed Laboratory of Neurophysiology. A true paterfamilias, Kuffler took with him four young faculty people then working independently in his Hopkins laboratory: David Hubel (from Walter Reed) and Torsten Wiesel, the two "brain boys," as they were called; and Ed Furshpan and David Potter, the "two membrane boys." These five were soon joined by a sixth colleague, the enzymologist Ed Kravitz, whose presumed function it was to explain it all in the universal language of biochemistry.

Harvard initially responded to Kuffler's brilliant recruitment effort by demoting Hubel and Wiesel from the assistant professorships they had enjoyed at Johns Hopkins to a nonprofessorial rank. But Harvard had found its match in Kuffler. Over the next 10 years, in his own quiet way, Kuffler turned the Laboratory of Neurophysiology at Harvard, consisting of one professor and five postdoctoral fellows, into a Department of Neurobiology, the first in the country (and probably in the world)—a department ultimately consisting, strangely enough, of six tenured professors: Kuffler, Hubel, Wiesel, Furshpan, Potter, and Kravitz.

In forming the Department of Neurobiology, Kuffler surmounted the conventional divisions that had separated the subdisciplines of the neurosciences and succeeded in establishing a unified discipline that represented the various subfields. In their training of students, and in developing neurobiology courses at the Harvard Medical School as well as summer courses at Woods Hole and the Salk Institute, the Harvard neurobiology faculty emphasized problems that were to become central to the field: How do neurons communicate with one another? How do they form patterns of interconnections? How do those patterns of interconnections give rise to perception?

Thus, what formed the initial basis of modern academic neuroscience—the fusion of the traditional, albeit disparate, neurobiological strands into one department—was born out of Hopkins' gift to Harvard. The formation of the first Department of Neurobiology was soon followed by the formation of similar departments at other universities, which led naturally to the formation of a new national society, the Society for Neuroscience. Until 1968, the national representation of scientists working on the brain had been fragmented: Neurophysiologists were associated with the American Physiological Society, neuroanatomists with the American Anatomical Association, biochemists with the American Biochemical Society, and psychologists with the American Psychological Association. In 1968, under the leadership of the psychologist Neil Miller, the biochemist Ralph Gerard, and the neurophysiologist Vernon Mountcastle, the Society for Neuroscience was founded. Its first meeting in Washington, DC, attended by approximately 600 people, proved to be a remarkable success and presaged the extraordinary explosion of interest in the field, which by the 1990s numbered well over 20,000 active members.

The First *Annual Review of Neuroscience*

A decade later, in 1978, the first volume of the *Annual Review of Neuroscience* appeared. This was a clear recognition that this new synthesis of disciplines had matured and could stand on an equal footing with other fully mature basic science disciplines, for each of which there was a long-standing *Annual Review* series. This emphasis on coherent multidisciplinary approaches to brain function produced dramatic changes in the field. It encouraged scientists to focus on problems of central importance to the biology of the brain, rather than on the problems accessible to one or another method in which the scientist was trained. Thus, people became concerned with signal transduction in neurons, with the mechanisms of cell-cell communication, with cell-cell recognition and the development of the nervous system, with the neurobiological basis of perception and action, with the genetic determinants of normal and abnormal behavior, and with the mechanisms of learning and memory.

In addressing these central questions in brain research, neuroscientists began to employ the techniques of several disciplines, using whatever methods were optimal for attacking specific problems. This focus on problems rather than on methods made great demands on scientists. Fortunately, the merger of the subdisciplines made neuroscience so attractive that it began to recruit to its ranks talented students and young scientists from other areas, including molecular biology, genetics, and immunology. These scientists brought with them new approaches to some of the traditional problems of neural science—approaches that incorporated molecular, biological, and genetic analyses, which greatly helped neural science to advance into the next phase of its development.

The initial volume of the *Annual Review of Neuroscience* not only signified the success of the Kufflerian synthesis, it also heralded the next phase: the emergence of molecular neuroscience, the application of recombinant DNA technology and molecular genetics to neurobiological problems, and the unification, within a common intellectual framework, of neuroscience with the rest of the biological sciences.

With the development of molecular neuroscience, the discipline as a whole began to overcome the intellectual barriers that had separated the study of the brain from the rest of biological science, barriers that had existed because the earlier language of the brain sciences had been based heavily on neuroanatomy and electrophysiology and only modestly on the more universal biological languages of biochemistry and cell and molecular biology. As a result, until about 1980, most molecular biologists felt that merely being interested in the central question posed by neurobiology—how does the brain operate?—was insufficient motivation for work on the brain, and that even to begin working on the nervous system required an extensive knowledge of neuroanatomy or electrophysiology. But with time it became clear that although a thorough understanding of the issues confronting the study of the brain was clearly needed, one could begin to work on molecular aspects of many problems without being intimidated by the for-

midable facts of electrophysiology or overwhelmed by the wealth of fine details in brain anatomy. Because in principle the methodologies of modern biology can be applied to any system of interest, some gifted newcomers made interesting contributions to neurobiology by selecting systems in which the anatomical and physiological details were either strictly limited or at least fairly straightforward.

As progress accelerated, the barriers that traditionally separated neurobiology from the rest of biology were progressively broken down. In particular, neuroscientists came to appreciate that such problems as transmitter release from presynaptic axon terminals are, in principle and often in detail, an aspect of a broader biological phenomenon, in this case vesicle exocytosis. This, in turn, led to the realization that some neuroscience problems can be more profitably studied in nonneuronal cells, such as in yeast or in frog oocytes. Moreover, it soon became apparent that the conservation of molecules and mechanisms applies not only to cellular function but also to whole biochemical and developmental pathways.

During the past decade, as our understanding of the molecular biology of neuronal signaling has rapidly advanced, the attention of neuroscientists has become increasingly focused on systems of nerve cells and on the neural underpinnings of complex behavior and its disorders. This has slowly led to a merging of systems neuroscience with cognitive psychology. This merger has given rise to the new field of cognitive neuroscience—the attempt to combine the techniques and experimental approaches of the brain sciences with those of the behavioral sciences so as to examine the biological bases of higher cognitive function. Although this movement had its origin in the 1980s, its impact is only now beginning to be felt.

The Emergence of Cognitive Neuroscience

The coming together of the traditional but historically distinct subdisciplines of brain research to form the coherent interdisciplinary field of neuroscience represented the first key synthesis in the formation of the modern discipline. The impact of molecular biology and molecular genetics on neuroscience represented the second step. The third logical advance toward the modern synthesis was the merger of neuroscience with cognitive psychology.

The first step in this direction was the extension in the 1950s and 1960s of the concepts of cellular physiology to systems neuroscience, a development that was epitomized by the work of Mountcastle on the functional organization of the mammalian somatic sensory system and of Hubel and Wiesel on the organization of the visual system. However, the time was not yet ripe to focus on higher mental functions, including conscious attention, sensory perception, the organization of motor action, learning and memory, and language. Cognitive neuroscience, which brought together the study of mental activity with the biology of the brain, only began to emerge in the 1980s, fully two decades later. This new synthesis came from the developments of several technical and conceptual advances whose importance can best be appreciated in an historical context.

In its early years, experimental psychology was concerned primarily with the study of sensation, but by the turn of the century the dominant interests of psychologists had turned to the study of such behaviors as learning and memory. The discovery of simple experimental means for studying learning and memory—first in humans by Ebbinghaus in 1885, and a few years later in experimental animals by Pavlov and Thorndike—led to a rigorous empirical school of psychology called behaviorism. Behaviorists, such as Watson and Skinner, argued that behavior could be studied with the same precision achieved in the physical sciences, but only if students of behavior abandoned speculation about what goes on "in the mind" and focused instead on observable aspects of behavior. For behaviorists, unobservable mental processes, especially anything as abstract as perception, attention, or memory, were deemed inaccessible to scientific study. Instead, they concentrated on examining—objectively and precisely—the relationship between specific physical stimuli and observable responses in intact animals. Their early and dramatic successes in rigorously studying simple forms of behavior and learning encouraged them to treat all processes that intervene between the stimulus (input) and behavior (output) as inaccessible and even irrelevant to a scientific study of behavior.

The appeal of behaviorism was such that by the 1950s, many psychologists came to accept its most radical position, namely that observable behavior is all there is to mental life. As a result, the scientific concept of behavior was largely defined in terms of the limited techniques used to study it, and inevitably some of the most fascinating features of mental life were excluded from study.

In the 1960s, the founders of cognitive psychology—Ulric Neisser at Cornell, Noam Chomsky at MIT, Herbert Simon at Carnegie Mellon, George Miller and Jerome Bruner at Harvard, and others—began to convince the scientific community of the point (by then rather obvious) that our mental life was, in fact, much richer than that defined by the behaviorist framework (for example see Neisser 1967). Building on earlier thinking of Gestalt psychologists and European neurology and neuropsychology, the cognitive psychologists emphasized that our knowledge of the world is based on our biological apparatus for perceiving the world, that perception is a constructive process that depends not only on the information inherent in a stimulus but also on the mental structure of the perceiver. Thus, cognitive psychology emphasized that to understand perception, one needed to do more than specify the physical nature of the sensory input and the observable behavioral output. To evaluate how a stimulus leads to a particular behavioral response, it was essential to analyze also the intervening processes by which sensory information was transformed into perception, memory, and action.

In redirecting scientific attention to mental operations, cognitive psychologists focused on information processing, on the flow of sensory information from peripheral sensory receptors to its eventual actions within the brain that lead to information storage (memory) or motor activity (behavior). The cognitive approach to behavior assumed that each perceptual or motor act has an internal representation within the brain: a representation in neural activity. But emphasiz-

ing internal representations as an essential component of behavior is one thing, studying it was another. In the late 1970s, most mental processes were not amenable to direct experimental analysis. Over the past two decades, several different approaches aimed at characterizing the neural substrates of information processing states have come together, and in the past decade, they have led to the maturation of cognitive neuroscience.

An important first step in this direction came from the work of Ed Evarts on the neural correlates of movement and, subsequently, from the studies of Vernon Mountcastle and his colleagues on somatic sensation. Their work served to establish that it is possible to approach the question of the internal representations of specified behaviors by studying the activity of single neurons in the cerebral cortex in intact, awake, behaving primates that are trained to do particular motor or perceptual tasks. In their work, and later in the work of Robert Wurtz, Michael Goldberg, Anthony Movshon, William Newsome, Charles Gross, Robert Desimone, John Maunsell, Tom Albright, and others, these cellular studies in monkeys led to the ability to correlate cognitive processes, such as attention and decision-making, with patterns of firing of individual cells in specific brain regions. For example, the work of Mountcastle, Wurtz, Gross, and Movshon opened up for functional study the vast regions of the extrastriate and related cortical areas, and Goldberg and Desimone developed experimental approaches that allowed selective attention to be studied on the cellular level. This changed the way behavior is studied both in experimental animals and in humans, as positron emission tomography and functional magnetic resonance imaging made it possible to explore brain activity in alert subjects.

The early developments in cognitive neuroscience stimulated a renewed interest in the behavioral analysis of patients with lesions of the brain that interfere with mental functioning. As first clearly shown by Pierre Paul Broca in 1863, in his studies of the role of the left frontal cortex in the expression of language, lesions of specific regions of the brain can cause specific cognitive deficits. From the time of Broca until the development of modern imaging methods, the approach of correlating brain lesions with behavior yielded a rich harvest of information about functional localization within the human brain, which in retrospect is probably the major contribution of clinical neurology to brain science. This field had remained strong in Europe and in Canada (as evident, for example, in the contributions of Wilder Penfield and Brenda Milner to the study of memory), but it was largely neglected in the United States, with some notable exceptions, such as the work of Lukas Teuber and Suzanne Corkin at MIT, of Norman Geschwind at Harvard, of Tony and Hanna Damasio at Iowa, and, more recently, of Larry Squire and his colleagues at San Diego and Daniel Schacter at Harvard. The development first of computerized axial tomography and later positron emission tomography and, most recently, functional magnetic resonance imaging provided, for the first time, direct access to the functioning of the intact living human brain. And when combined with well-conceived behavioral experiments, as in the work of Posner, Raichle, and their colleagues in the United States and of Zeki, Frac-

kowiak, and their associates in England, an entirely new range of experimental possibilities opened up (for review, see Posner & Raichle 1994). A further extension of this approach combines the identification of areas in the human brain that are active under specific behavioral conditions with parallel studies of neuronal activity in the corresponding areas in the brains of conscious monkeys carrying out essentially the same behavioral task. This combined approach in monkeys and humans promises to be a powerful tool for analyzing a variety of complex higher-brain functions.

Computer science also has made a distinctive contribution to cognitive neural science. Models of associative memory, such as those of Hopfield and others, and the development of the back propagation learning algorithm by Rumelhart and Hinton have shown, in principle at least, that complex behaviors could arise from the intrinsic properties of neural nets. Computers also have made it possible to model in some detail the integrative properties of individual neurons and to some extent the activities of large populations of neurons, making it possible to begin to test ideas about the role of specific components of the brain in particular behaviors. To understand the neural organization of such complex behaviors as perception or speech requires an understanding not only of the properties of individual cells and pathways but also of the network properties of functional circuits in the brain. Such network properties, although obviously dependent on the properties of individual neurons in the network, go well beyond these properties and provide for the emergent properties that are commonly referred to as higher brain functions. Computational approaches along these lines, and especially when combined with psychophysics, as in the work of Terry Sejnowksi, Steven Lisberger, Richard Andersen, Emilio Bizzi, and Tony Movshon, have been helpful in specifying what particular systems are capable of doing and in suggesting how the properties of the constituent cells and pathways may account for the properties of the system as a whole.

The Impact of Molecular Genetics on Neuroscience

Of all the developments that have contributed to neuroscience in the past two decades, whether technical or conceptual, none has had a greater impact than the application of molecular genetics to the problems of neuronal signaling and neural development. It is becoming increasingly evident that in the near term, the extension of genetic approaches to clinical neurology and psychiatry holds greater promise than any other approach for our understanding of the etiology of various disorders and for their diagnosis. It is appropriate, therefore, to review the history of modern genetics and the emergence of the subdiscipline that is often referred to as behavioral genetics, and to describe some examples of the way in which genetics is beginning to inform, and may in time transform, neurology and psychiatry.

The early history of genetics was characterized by the initial discovery and later rediscovery of Mendel's laws. This was followed by the classical era, in

which the significance of mutations and the linear arrangement of genes along chromosomes was established by Thomas Hunt Morgan and his school. Not long afterward, Herman Muller found that specific mutations could be produced by X-ray irradiation, which made it easier to analyze their functional significance. Of particular interest, in the present context, was the recognition during this period of the usefulness of model organisms, and especially of the fruit fly *Drosophila melanogaster,* with its four chromosomes, its short generation time, and the relative ease with which mutant phenotypes can be recognized.

In the mid-1960s, Seymour Benzer began to focus his attention on a search for mutations in *Drosophila* that clearly affected the animal's behavior (see Benzer 1967, 1973). Because flies display a wide spectrum of fairly sophisticated behaviors, ranging from phototropism, circadian rhythms and mating, to simple forms of learning and memory, Benzer's approach proved to be singularly effective and paved the way for the continuing use of *Drosophila* both for behavioral genetics and for studies of ion channels, second messenger systems, and, most strikingly, the analysis of neural development.

At about the same time, Sydney Brenner introduced the soil nematode, *Caenorhabditis elegans,* as a model system for developmental and neurobiological studies (Brenner 1965, 1973). The worm is a self-fertilizing hermaphrodite, so mutants give rise to progeny that are homozygous at almost all loci. Their generation time is even shorter than that of *Drosophila*—~3.5 days—and each parent can give rise to 250 or more progeny. The worm is very small—approximately 60 μm × 1 mm—so up to 10^5 individuals can be maintained in a Petri dish. The adults have only 816 somatic cells, of which 302 are neurons. The complete lineage and fate of every cell is known, and the three-dimensional morphology of the entire nervous system, including the position, size, shape, branching pattern, and interconnections of the cells and their processes, has been mapped at the electron micrographic level. This kind of reconstruction has permitted the detection, down to the synaptic level, of morphological changes caused by mutant genes. Many behavioral mutants have been identified. Among them are many affecting chemosensation, which have provided a valuable parallel to the genetic studies of chemosensation in mammals.

C. elegans permits the screening of thousands of putative mutants and thus the recovery of more and rarer mutations than in any other metazoan organism. Thus, it is possible to achieve a level of genetic control rivaling that in yeast and bacteria. Most important, nearly all the genes identified in mutant screens of *C. elegans* and *Drosophila* have been found to be present in distant relatives such as ourselves. Now that all of the more than 19,000 genes of the *C. elegans* genome have been cloned and sequenced, it is impressive that 70% of the genes so far encountered in humans have orthologs in the worm.

Mice have proven advantageous for neuroscience, as for other disciplines, as a mammalian experimental system that nevertheless has some of the advantages of the invertebrate systems. Mice have been the subject of genetic research as long as fruit flies, and spontaneous neurological mutations such a weaver and

reeler have long served as animal models of disease and provided some of the key insights into cortical development. They were studied by Richard Sidman and by Pasko Rakic as early as the 1960s. However, only recently have advances in genetic technology made mouse genetics powerful enough to be used routinely to study the behavioral effects of mutations in specific genes. The reason for this delay is obvious: Although mice represent a relatively tractable vertebrate model, compared to invertebrates they are large, their generation time is long, and their litter size is small. In addition, their genome is complex. Nevertheless, the development by Richard Palmiter, Ralph Brinster, and others of methods for expressing individual genes (transgenic mice), and by Mario Capecchi and Oliver Smithies of the technique of gene targeting by means of homologous recombination in embryonic stem cells, has permitted the genetic engineering of new mouse mutants. The result has been a steady stream of transgenic mice to examine gain-of-function and of knock-out mutants designed to examine loss-of-function of individual genes. Although the production of mutants by this technique is relatively slow (~1 year), it has given a significant boost to the attractiveness of mice as an experimental system closer to humans. More recently, the advent of improved gene mapping techniques has permitted the development of extensive and rapidly expanding genetic and physical maps. These have opened up new possibilities for mapping genes governing natural polymorphisms in behavior.

Although humans lack most of the experimental advantages of worms, flies, or mice, they offer the advantage that human behavior is interesting and diverse, and that we have an extraordinary interest in understanding human behavior and in coming to grips at last with several rather intractable neurological and psychiatric disorders. The ability to study human genes has made a quantum leap in the past decade with the introduction of DNA markers to facilitate gene mapping. Currently, the principal approach is to use genetic polymorphisms to identify loci in which to search for mutations that, once mapped, can be cloned and characterized. However, the anticipated sequencing of the complete human genome within the next few years and the availability of over a million expressed sequence tags promises to accelerate dramatically the ability to identify pathological mutations.

NEUROLOGICAL DISEASES ILLUSTRATE THE SUCCESS IN ANALYZING MONOGENIC DISORDERS

The early work on the genetics of various mouse mutants that affect the nervous system suggested that within a decade or two, the genetic basis of a large number of human neurological disorders would begin to be understood. That this prediction has proved to be correct is evident by the growing number of reports of the discovery of new genes or new genetic loci for a wide range of neurological abnormalities. Indeed, hardly a month goes by without journals such as *Science*,

Cell, *Proceedings of the National Academy of Sciences*, *Nature*, and *Nature Genetics* reporting such discoveries. This is hardly surprising given that a significant proportion of the human genome is devoted to genes that are expressed, either uniquely or at least predominantly, in the nervous system.

Progress in analyzing genes important for neurological disorders has been so rapid and so dramatic that it is impossible to describe more than just a few examples. These examples should serve not only to indicate the importance of this approach, but also to suggest that within the next generation the entire field of neurology may be transformed profoundly. Indeed, it is likely that neurology will be transformed even more radically in the next decade by genetic analyses than it has been transformed in the past decade by the informative analysis made possible by magnetic resonance imaging. We make this claim because we believe that, in time, molecular genetics will provide neurology with an entirely new intellectual framework, a new nosology, based not on symptomatology or the traditional neuropathological classifications of infectious, vascular, neoplastic, and other disorders but on the underlying molecular and cellular mechanisms. Some first steps toward this are considered below in discussions about the so-called channelopathies and mitochondrial disorders, many of which could not have been recognized until the relevant genes had been identified, cloned, and sequenced and specific mutations recognized. It may be premature to suggest that before long clinicians will think in terms of the dysfunction of particular neuronal organelles (such as lysosomes, the endoplasmic reticulum, or the cytoskeleton) or of particular classes of molecules (receptors, ion channels, intracellular signaling pathways, or transcription factors). But this is clearly where the direction of the future lies, and also the direction in which new therapies are likely to be focused.

A major advance in the study of human genetic disorders occurred in the early 1980s with the development of restriction fragment length polymorphism analysis. Until that time, the genetic markers used to track genes and their mutations in human chromosomes were based solely on variations in coding regions of DNA, expressed ultimately as proteins. The common markers were blood group antigens, certain enzymes, and the antigens of the histocompatibility complex. However, DNA encoding gene products probably accounts for less than 10% of the human genome; more than 90% of the genome contains noncoding sequences, previously referred to as junk DNA. In 1980, Botstein et al (1980) realized that polymorphic sites could be recognized using restriction endonucleases. They pointed out that in the limit, single–base pair changes, which are genome specific and are tied to how closely individuals are related, can be detected by changes in restriction digest patterns. And it is important that these single–base pair changes are diagnostic even when the nucleotide changes occur in noncoding regions. These restriction fragment length polymorphisms (RFLPs) allowed saturation of the human genome with markers in noncoding as well as coding DNA regions, and this broad coverage made it easier to pinpoint the chromosomal loci of inherited diseases. Indeed, even before the report by Botstein and his colleagues, Kan

and colleagues (Kan & Dozy 1978, Kan et al 1980) were able to show how RFLP analysis could be used for the prenatal diagnosis of a clinical disorder (in this case, sickle-cell anemia).

Duchenne's and Other Muscular Dystrophies

Kay Davies and her colleagues used the RFLP approach to identify the locus for Duchenne's muscular dystrophy, an X-linked disease that affects young males, and found restriction length polymorphisms that linked the disease to the area flanking the Xp region of chromosome 21 (Forrest et al 1988). Uta Francke and her colleagues (1985) next identified a large deletion of the region around Xp21 in a patient with five different X-linked conditions, one of which was Duchenne's dystrophy. This allowed Louis Kunkel and his associates to clone the Duchenne's gene (Hoffman et al 1987, Hoffman & Kunkel, 1989). They found the Duchenne's gene to be about 2.5 million base pairs in length, the largest gene then characterized. This one gene alone accounts for 1% of the X chromosome and almost 0.1% of the total genome. The protein encoded by the gene, dystrophin, has homologies to alpha actinin and spectrin, two cytoskeletal proteins located on the inner surface of the plasma membrane. Dystrophin is linked at its carboxyl terminus to the cytoskeletal actin beneath the sarcolemma. Boys with Duchenne's dystrophy lack dystrophin completely. Boys with Becker's dystrophy, a milder form of the disease, have some functional protein but in subnormal amounts. In cases of Becker's dystrophy, the location of the deletions within the gene clearly influence the phenotype: In general, mutations at either end of the dystrophin gene give rise to the most severe forms of Becker's dystrophy, whereas mutations in the central region produce less-severe symptoms (Figure 1) (for review, see Worton 1995).

The discovery of dystrophin marked a turning point in the study of neuromuscular disorders. First, the discovery suggested that in the fullness of time it should be possible to delineate precisely all monogenic disorders of the nervous system. And second, it was evident that information and ideas gleaned from studies of such diseases were likely to provide valuable insights into basic problems in neurobiology. Together these conclusions spurred the search for other genes that are important in neurological diseases, including the genetic bases of other, more rare types of muscular dystrophy.

It is interesting to note that the discoveries of these more rare forms of muscular dystrophy have owed as much to traditional biochemistry as they have to molecular genetics (Figure 2). The key discovery was the finding by Kevin Campbell and his colleagues that dystrophin is only one component of a much larger complex of glycoproteins associated with the sarcolemma, collectively referred to as the dystroglycan complex, which serves to link the sarcoplasmic cytoskeleton to the extracellular matrix. The complex consists of three components: the dystroglycans, the sarcoglycans/sarcospan complex, and the dystrophin-syntrophins. All these components were identified initially in immunoprecipitation and protein

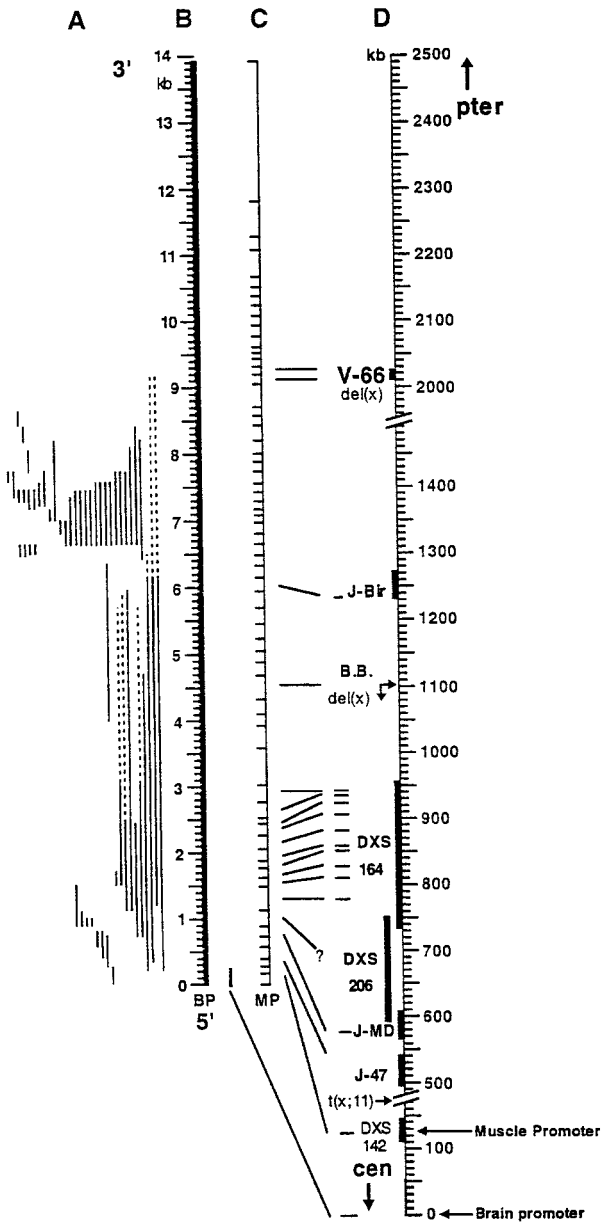

Figure 1 Deletion mutations in the dystrophin gene leading to Duchenne's and Becker's muscular dystrophy. (*A*) Deletions found in 53 patients with either Duchenne's or Becker's muscular dystrophy. (Vertical lines represent single patients, the region indicated being the exons missing from the cDNA. Dotted lines indicate that the precise limits of the deletion mutation were not known for that patient.) (Redrawn from Koenig et al 1987, Nobile & Marchi 1994.) (*B*) A map of the cDNA is shown and units are in thousands of base pairs (kb). (*C*) A representation of individual exons, which together make up the complete mRNA. (Horizontal lines) The location of certain exons within the genomic DNA (*D*). (*D*) A schematic representation of the gene (genomic DNA), with the centromere of the X chromosome oriented toward the bottom (cen) and the tip of the short arm of the X on the top (pter). Units are thousands of base pairs (kb) of DNA. (Bold vertical lines) Regions of contiguous DNA that had been cloned by 1987, with locus designations indicated. The first exons of the gene were isolated from the region marked DXS 164. (From Hoffman 1997.)

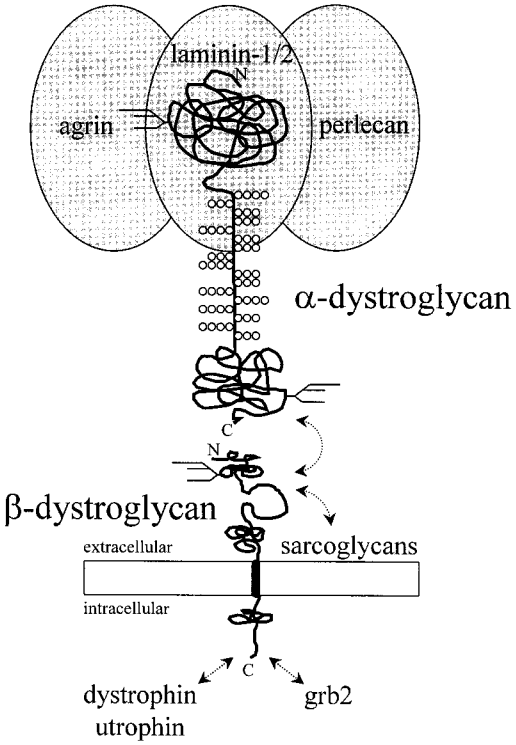

Figure 2 Location and organization of dystrophin and dystrophin-associated proteins in the myofiber. The dystrophin membrane cytoskeleton complex includes dystrophin as a major structural component on the intracellular face of the plasma membrane, where it is thought to form a mesh-like oligomeric structure based on antiparallel dimers or tetramers. A series of dystrophin-associated proteins interact with dystrophin and mediate its attachment to the membrane and other proteins. Three groups of dystrophin-associated proteins have been identified to date. The sarcoglycan complex includes three or four distinct transmembrane proteins, which are called α-, β-, and γ-sarcoglycan based on the order in which they were identified. Primary deficiencies of each of these proteins have been shown to cause some cases of inherited muscular dystrophies with a clinical phenotype similar to Duchenne's or Becker's muscular dystrophy. The dystroglycan complex is formed by the proteolytic cleavage of a single polypeptide into a transmembrane β-dystroglycan, and a larger extracellular α-dystroglycan. The dystroglycans mediate the attachment of dystrophin to laminin, the major component of the myofiber basal lamina. Laminin 2 (laminin) is a heterotrimer consisting of a large central protein (α2 laminin, also called merosin), and two smaller subunits (β1 and γ1 laminin) (outer arms of molecule). Deficiency of α2 laminin causes approximately 30% of cases of congenital muscular dystrophy. (From Henry & Campbell 1996.)

purification experiments; only later were the relevant genes cloned and sequenced. What is particularly important in the present context is the discovery that mutations affecting different components of the dystroglycan complex are responsible for specific muscular dystrophies. The first of these to be identified was severe childhood autosomal recessive muscular dystrophy, which was shown to be caused by mutations in the gene for α-sarcoglycan (also known as adhalin). Shortly thereafter it was found that mutations in the gene for β-sarcoglycan are responsible for a form of autosomal recessive limb girdle muscular dystrophy. And it is interesting that the absence of β-sarcoglycan is accompanied by the loss of the other sarcoglycans (α, β, δ). More recently, other forms of limb girdle muscular dystrophy have been found to be associated with mutations in each of the other sarcoglycan genes (Straub & Campbell 1997). Indeed, it now seems

likely that in time, mutations in each component of the three subcomplexes will be found that cause some form of muscular dystrophy, and it is already clear that mutations affecting certain of the associated extracellular matrix components can give rise to yet other muscular dystrophies. For example, mutations in the gene for laminin $\alpha 2$ have been found to cause congenital muscular dystrophy in Caucasians (Allamand et al 1997).

Huntington's Disease

Although the discovery that mutations in the dystrophin gene are the cause of Duchenne's muscular dystrophy was historically the first major contribution of genetic neuroscience to neurology, the discovery of the gene responsible for Huntington's disease (HD) is perhaps its most celebrated contribution.

HD is an autosomal dominant disease characterized by progressive motor dysfunction and cognitive impairment. The motor symptoms include involuntary choreiform movements, increasing rigidity, abnormal ocular saccades, and disturbed balance and coordination; the cognitive symptoms include personality changes, depression, and dementia. After a period of weight loss, patients generally advance to a vegetative state, and death usually occurs 15–20 years after the onset of symptoms. The disease usually manifests itself in midlife, often after the reproductive years. Pathologically, it is characterized by marked neuronal cell death primarily in the striatum but also affecting the cerebral cortex.

The study of HD exploited another valuable tool in human genetics, the existence of extended families. In the early 1980s, Nancy Wexler and her colleagues began studying an extended family whose members were descendants of a woman who lived near Lake Maracaibo, in northwestern Venezuela, early in the nineteenth century. This woman inherited the disease from her father, an English sailor, who carried a mutation in the gene, and most of her descendants married and remained near Lake Maracaibo, where there are now more than 3000 living relatives, of whom over 100 suffer from HD. In addition, there are more than 1000 children, each of whom has a 50% chance of inheriting the disease. In 1983, Gusella & Wexler began to screen DNA samples from members of this family and, using RFLPs, succeeded in localizing the gene for HD to near the end of the short arm of chromosome 4 (Gusella et al 1983).

A full decade later the gene was finally isolated and shown to encode a protein of 350,000 kDa, now called huntingtin (Huntington's Dis. Collab. Res. Group 1993). This protein is expressed both in the brain and in a variety of other tissues. The exact function of huntingtin is still not known, but studies indicate that the normal protein is located in the cytoplasm. Based on reports of defective electron transport activity in postmortem brain tissue (Parker et al 1990, Brown 1997), a primary candidate underlying the cellular pathology of HD was at first thought to be mitochondrial dysfunction, but it is now clear that huntingtin does not colocalize with mitochondria.

What proved particularly important, however, was the finding that the first exon of the gene contained large numbers of repeats of the trinucleotide sequence CAG, encoding the amino acid glutamine. In normal subjects there are fewer than 40 such CAG repeats, but in patients with HD the number of repeats is often significantly greater. Individuals with more than 40 CAG repeats in either copy of the gene invariably suffer from HD, and individuals with more than 70 such repeats usually develop HD as juveniles (Figure 3). Once the repeat expands to more than 40 copies, it becomes increasingly unstable. Of particular interest is the finding that the number of repeats can increase from generation to generation. This accounts for the clinically observed phenomenon of "anticipation," characterized by the earlier onset (and sometimes greater severity) of the disease in succeeding generations, as dramatically observed in the Lake Maracaibo population. "Anticipation" had seemed to violate Mendelian principles, but the finding of the increased number of repeats between parents and their offspring has supplied a satisfying explanation for the phenomenon (Figure 4).

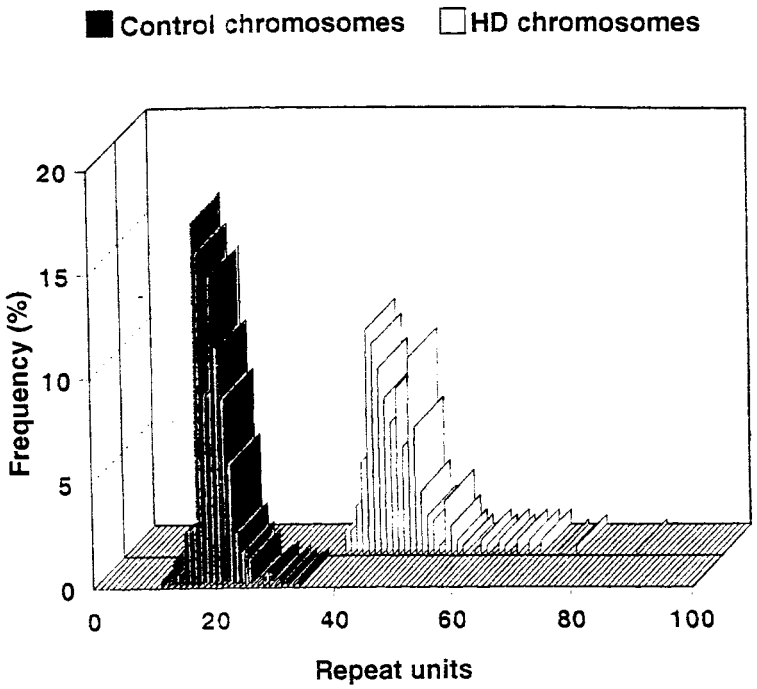

Figure 3 Comparison of $(CAG)_n$ repeat length on 425 Huntington's disease (HD) chromosomes from 150 families compared with $(CAG)_n$ repeat from 545 control chromosomes. HD chromosomes showed 37–86 repeats; normal chromosomes had 11–34 repeats. (From Duyao et al 1993.)

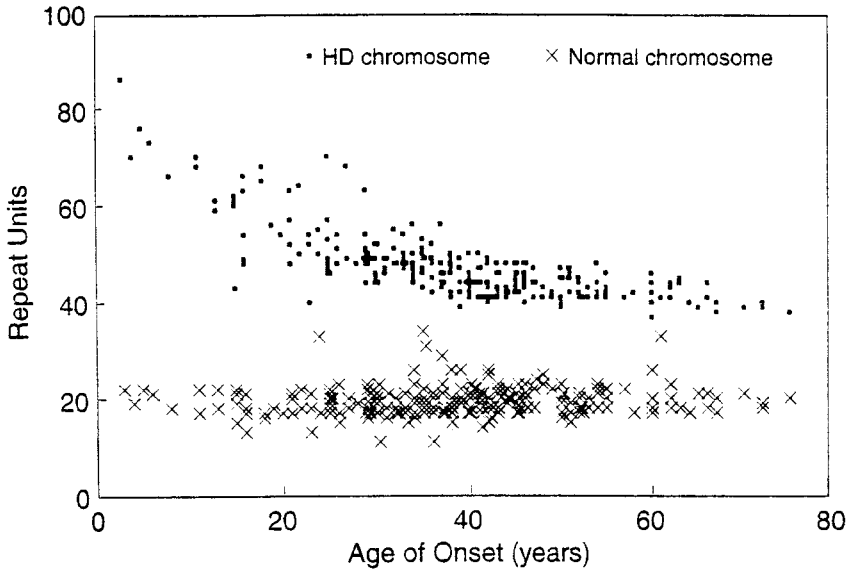

Figure 4 Relationship of Huntington's disease (HD) gene CAG repeat length to age of onset, and number of CAG repeats on the normal allele in 234 individuals with clinical HD. There is a strong correlation between repeat length, early age of onset, and diagnosis of clinical disease by age 20. The repeat length is not a strong indicator of age of onset in those without signs of illness at age 20 and, consequently, is not relevant to most people seeking presymptomatic testing. (From Duyao et al 1993.)

Other Trinucleotide Repeat Disorders HD was not the first genetic disorder in which trinucleotides repeats were observed. They were first reported when the gene for Fragile X mental retardation was sequenced (Kremer et al 1991, Verkerk et al 1991). Subsequently, a number of other hereditary disorders of the nervous system have been found to be associated with similar repeats, and collectively they form the largest group of dominantly transmitted neurological disorders (for review, see Paulson & Fischbeck 1996, Ross 1997, Zoghbi & Orr 2000).

We do not, in this overview, consider all the trinucleotide repeat disorders. We limit ourselves to summarizing the major features of the non-HD disorders [based largely on the review by Paulson & Fischbeck (1996)].

There are eight trinucleotide repeat disorders known, and they fall into two distinct groups, based primarily on the nature of the trinucleotide repeat. Type 1 disorders involving CAG repeats (such as that in HD) include spinal and muscular atrophy (Kennedy's disease); spinocerebellar ataxia types 1, 4, 6, and 7; dentatorubral ataxia; dentatorubral-pallidoluysian atrophy; and Machado-Joseph disease. All these disorders are characterized by relatively modest numbers of CAG repeats (usually not exceeding ~90), with only moderate degrees of instability.

The CAG repeats fall within the coding region of the gene, they are translated as polyglutamine runs, and they seem to act to cause the associated disorders through a gain-of-function mechanism. Type II repeat disorders include Fragile X (FRAX A and FRAX E with CGG and GTC repeats, respectively) and myotonic dystrophy [with CTG repeats (Brook et al 1992)]. In these three disorders, the trinucleotide repeats are found in either the 5' or 3' untranslated regions of the gene and, hence, are not translated into protein. The numbers of repeats are also considerably larger, ranging from ~200–2000 in the case of fully developed Fragile X and myotonic dystrophy. Unlike type I disorders, whose effects are largely, if not exclusively, limited to the central nervous system, those of type II are widely manifest in many organ systems and are due not to altered protein function but to attenuated expression of the gene; in addition, they tend not to be progressive but instead reflect an early developmental disorder. In the case of Fragile X, in which the related protein, FMR 1, is not expressed (apparently as a result of the methylation of the expanded repeat), one clue to its possible mode of action is the presence within the protein of known RNA binding motifs (Warren & Ashley 1995). That these binding motifs are critical has been demonstrated dramatically in one severely retarded patient with a single point mutation in one of the RNA binding domains.

The Discovery of Neuronal Intracellular Inclusions in Huntington's Disease Although polyglutamine tracts can exist in a variety of configurations, the most common form is that of beta sheets consisting of six to eight residues per strand. This conformation suggested to Max Perutz & his colleagues (1994) that the glutamine repeats could act as a polar zipper—a type of molecular velcro—to which other copies of the same protein, or even other proteins, could bind. If this were true of the CAG repeats, it seemed likely that the affected proteins would be trapped. This would not only prevent the protein from functioning normally, it would also, by forming large aggregates, prove to be toxic to the cells. In the case of HD, it was postulated that the accumulation of mutant huntingtin protein in neurons might lead to the formation of toxic protein aggregates in certain neurons, similar to those observed in Alzheimer's disease or certain prion disorders. This has now been shown to be the case.

Mangiarini et al (1996) expressed the first exon from the mutant human huntingtin protein in transgenic mice and found that this is sufficient to cause a progressive neurological phenotype. Subsequently, Davies et al (1997) found that mice expressing this exon form multiple intranuclear inclusions and that these inclusions are made up of large aggregates of huntingtin protein bound to ubiquitin. The inclusions can be as large as a nucleolus and clearly visible in preparations stained with antibodies directed against either the N terminus of huntingtin or against ubiquitin. Davies went on to show that the formation of nuclear inclusions precedes the onset of symptoms. Because the affected mice displayed movement abnormalities similar to those seen in HD, neuropathologists were stimulated to go back and reexamine the brain tissue from patients who had died

from HD. This has led to the finding of huntingtin protein aggregates in the nuclei of striatal and cortical neurons. Similar inclusions have now also been found in a number of the other dominant gain-of-function neurological diseases caused by polyglutamine expansions. However, it is unclear why neurons in certain regions are severely affected whereas others carrying the same transgene (with the same polyglutamine tract) are unimpaired. Perhaps as Skinner et al (1997) have suggested, it is because the polyglutamines preferentially aggregate with other proteins that are either uniquely or predominantly expressed only in the affected neurons.

The identification of the CAG repeats and the nuclear inclusion bodies has opened up a new area of investigation, for both HD and other dominant gain-of-function diseases, that promises to unify the study of such triplet repeat diseases. For example, Ross and his colleagues created lines of mice containing the N-terminal portion of the truncated HD gene, which contains an expanded CAG tract with 82 repeats. All the lines developed neurological signs between two and six months of age. The signs included tremor, uncoordinated movements, and shortened lifespan—typically 5–10 months—compared to about 2 years for the normal mouse (for review, see Ross 1997).

Huntington's Disease Can Be Modeled in Transgenic Flies and in Mammalian Cells in Culture Larry Zipursky and his colleagues have developed a *Drosophila* model of HD by taking the amino-terminal fragment of the human huntingtin protein containing tracts of 2, 75, and 120 repeating glutamine residues and expressing the fragment in photoreceptor neurons of the compound eye of flies (Jackson et al 1998). In these photoreceptor neurons, the polyglutamine-expanded huntingtin induced neuronal degeneration much as it does in human neurons. The age of the onset and severity of the neuronal degeneration again correlated with the length of the repeat, and the nuclear localization of huntingtin again presaged neuronal degeneration. In contrast to other cell paradigms in *Drosophila,* the coexpression of the viral antiapoptotic protein p35 did not rescue the cell death phenotype induced by the polyglutamine-expanded huntingtin.

The cell death induced by the expression of the mutant protein shares some but not all morphological features of programmed cell death, or apoptosis. In *Drosophila,* programmed cell death can usually be blocked by the expression of the antiapoptotic protein p35, a broad specificity inhibitor of caspases, and this has been found to be the case in other forms of photoreceptor degeneration in *Drosophila* mutants. Jackson et al (1998) evaluated the effect of p35 in the photoreceptor cell death induced by the expanded polyglutamine repeats but were not able to rescue any of them. Their data, therefore, suggest that the cell death induced by huntingtin may proceed along a killing pathway different from that of apoptosis. However, an analogous fly model has been obtained by Warrick et al (1998), who expressed in *Drosophila* a polyglutamine-expanded fragment of the human Machado-Joseph disease (MJD) protein involved in spinocerebellar ataxia type 3. Here again, repeat length–dependent degeneration occurred, with

progressive nuclear accumulation of the expanded repeat-containing protein. However, Warrick et al (1998) were able to obtain some rescue of the degeneration by coexpression of the antiapoptotic p35 protein. As these two studies make clear, the cellular mechanisms for human polyglutamine repeat disease are conserved in invertebrates, and because of the rapidity of the ensuing cell death, there is every reason to believe that these animal models will accelerate our understanding of the underlying mechanism.

Recently, Michael Greenberg and his colleagues have developed a new cellular model of HD (Saudou et al 1998). They transfected the mutant Huntington's gene into cultured striatal neurons and found that the gene induced neurodegeneration by an apoptotic mechanism, consistent with the idea that huntingtin can act in the nucleus to induce apoptosis. Blocking nuclear localization of the mutant huntingtin suppresses its ability to form intranuclear inclusions and to induce apoptosis.

Neurological Disorders Associated with Mutations in Mitochondrial Genes

It is becoming increasingly clear that genetic mutations affecting a variety of neuronal organelles can give rise to distinct neurological disorders. For example, the various storage disorders associated with specific defects in lysosomes (or the pathways that lead to the storage of materials in lysosomes) have been studied for more than 4 decades (for review, see the relevant chapters in Scriver et al 1995). But one class of genetic disorder that merits special mention here is that due to mutations in mitochondrial genes. These are of particular interest because of the unusual organization of the mitochondrial genome (for review, see Wallace 1999).

Human mitochondria contain many copies of a closed circular DNA genome that comprises some 37 different genes. These genes encode 13 polypeptide products (all of which are involved in the respiratory chain), 22 tRNAs, and 2 rRNAs. Because of the large number of mitochondria per cell, the copy number of these genes is high, averaging 10^3–10^4 genomes/cell. Two features distinguish mitochondrial genes from their nuclear counterparts: First, they are inherited almost exclusively from the mother; second, mutations may affect all or only a proportion of the mitochondrial genes in a cell, and in the latter case, whether the respiratory chain is affected or not appears to depend on a certain threshold level (usually ~60% or more) of the mutated DNA (Larsson & Clayton 1995).

Although the first mitochondrial disorder was reported more than 40 years ago (in a patient suffering from what is now referred to as mitochondrial myopathy—characterized by the presence of large numbers of distorted mitochondria), it is only in the past 12–15 years that mutations in mitochondrial genes have come to be recognized as the underlying disorder in a number of neurological conditions. The first of these to be understood was Leber's hereditary optic neuropathy, a maternally transmitted condition marked by the sudden onset of blindness, com-

monly affecting young men. Three different nucleotide transitions have been found to account for about 80–90% of patients with Leber's hereditary optic neuropathy, and the observed sex difference in the incidence of the disorder suggests that there may be an associated (recessive) X-linked factor (Bu & Rotter 1991).

A second mitochondrial disorder is Leigh's syndrome characterized by ataxia, spasticity, ophthalmoplegia, optic atrophy, and, later, marked degeneration of the basal ganglia. This condition, which principally affects infants, is commonly associated with a single mutation in the mitochondrial ATP6 synthase gene. Yet another disorder goes by the acronym MERRF (myoclonus epilepsy and ragged red fibers). It is characterized not only by the myoclonus epilepsy, but also by mental deterioration, ataxia, tremors, and muscle atrophy, and it is associated with mutations affecting the mitochondrial lysine tRNA, which inhibit mitochondrial protein synthesis. A similar tRNA gene mutation (affecting the leucine UUR tRNA) has been found in patients suffering from myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (or MELAS, for short).

This is by no means a complete account of the neurological complications that have been attributed to mitochondrial gene mutations, but it should serve to make the point alluded to earlier, that genetic analyses of this kind are likely in time to replace those that have been traditionally based on symptomatology. [For more detailed accounts, reference should be made to the reviews by Larsson & Clayton (1995) and Wallace (1999), but here it is worth noting that the surprising number of mitochondrial disorders is almost certainly attributable to the high mutation rate of mitochondrial DNA, which is said to exceed that of nuclear DNA by at least an order of magnitude.]

The Channelopathies: Diseases of Voltage- and Ligand-Gated Ion Channels

Because electrical signaling is critical for the functioning of neurons and muscles, it is not surprising that mutations in ion channel proteins should lead to disease. In fact, a group of molecular defects in voltage- and ligand-gated ion channels of muscle have now been identified that underlie the hereditary myotonias, the periodic paralyses, and a number of other neurological disorders. As a result, these disorders are now referred to as channelopathies—disorders of ion channel function.

Whereas our progress in understanding HD and most other heritable disorders of the nervous system has been relatively slow, the progress in elucidating the channelopathies has been rather dramatic. This rapid progress can be attributed directly to the large amount of basic scientific knowledge about channel function that was already on hand. To begin with, earlier electrophysiological studies of diseased muscle in both humans and experimental animals had identified abnormal ion channel currents and, hence, indicated candidate disease genes. Moreover, in contrast to the work on HD, which led to the discovery of a novel gene whose

normal function is still not known, many of the voltage-gated ion channels of skeletal muscle had already been cloned, expressed, and analyzed before they were recognized to be targets for disease, thereby circumventing the need to carry out laborious positional cloning. Finally, as in HD, animal studies and studies in cell lines have provided useful confirmation that the functional defects identified in mutant ion channels are sufficient to cause such specific neurological disorders as myotonia and periodic paralysis (for reviews, see Brown 1993a,b; Ptácek 1997, 1998).

Although the channelopathies are rare, they are of interest far beyond the myotonias and periodic paralyses. These particular disorders simply illustrate the dramatic effect on normal function that alterations in ion channels structure can produce. It would not be hard to imagine that other alterations in ion channel function in the brain, such as polymorphisms in various types of K^+ or Ca^{2+} channels, could contribute to subtle differences in human behavior, as evident in personality and character structure. However, currently, the better known channelopathies of neurological interest are the following (Figure 5).

Familial periodic paralyses are autosomal dominant disorders characterized by episodic periods of weakness or even paralysis, usually precipitated by muscular exertion and fatigue. Included in this group of disorders are (*a*) hyperkalemic periodic paralysis, which can be precipitated by excessive potassium intake and is now known to be due to mutations in muscle sodium channels (Figure 6) (for review, see Cannon 1996); and (*b*) myotonia congenita, one of a larger class of myotonias that can be either autosomal dominant (Thomsen's disease) or recessive (Becker's disease) and that is generally characterized by muscle hyperexcitability associated with mutations in muscle chloride channels (for example, see Steinmeyer et al 1994).

Hereditary hyperekplexia (Suhren et al 1966), marked by exaggerated startle responses and hypertonia, is another autosomal dominant disorder, now known to be due to mutations in the gene encoding the glycine receptor channel.

Episodic ataxias are marked by attacks of ataxia, nystagmus, and other motor disorders. One type (type I) is associated with mutations in a widely expressed potassium channel gene, whereas another (type II) is due to mutations in a calcium channel predominantly expressed in the cerebellum.

More recently, other channel disorders—including familial idiopathic epilepsy, a form of inherited epilepsy of newborns—have been found to be associated with mutations in a novel KQT-like potassium channel (see Stoffel & Jan 1998).

Familial hemiplegic migraine is a dominantly inherited condition characterized by typical migraine-like headaches associated with transient episodes of ataxia, nystagmus, and hemiparesis that may last for hours or days (Joutel et al 1993, Greenberg 1997). Currently, three distinct loci have been identified as being associated with familial hemiplegic migraine, two on chromosome 1 (Ducros et al 1997, Gardner et al 1997) and a third on the short arm of chromosome 19 (Ophoff et al 1996). The latter appears to account for about half the cases of familial hemiplegic migraine and a number of missense mutations have been identified in

Figure 5 Skeletal muscle disorders along the spectrum from myotonia to periodic paralysis. Muscle stiffness predominates in myotonia congenita and potassium-aggravated myotonia. Both stiffness and weakness are present in paramyotonia congenita and hyperkalemic periodic paralysis. Severe weakness without stiffness occurs in hypokalemic periodic paralysis. Chloride channel defects lead to myotonia, calcium channel mutations cause periodic paralysis, and sodium channel mutations may cause both stiffness and weakness. (From Cannon 1998.)

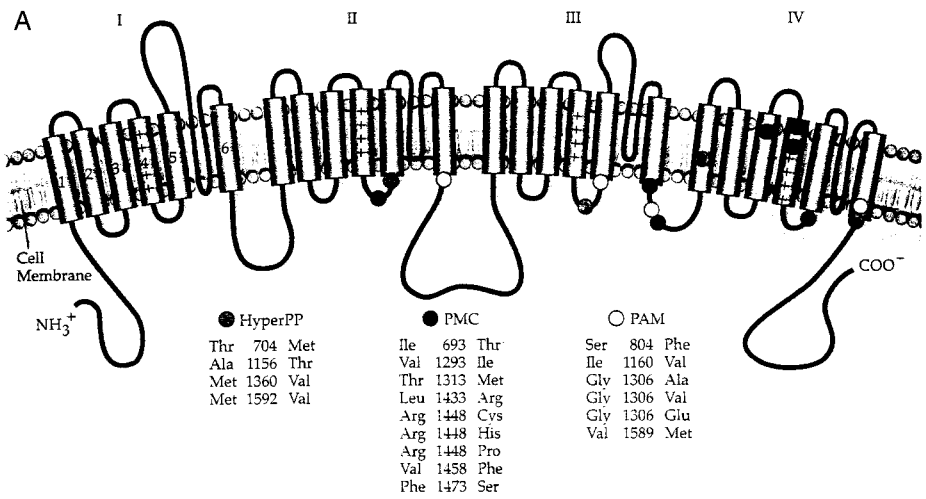

Figure 6 (*A*) Mutations in sodium channel α-subunit. Model of the Na channel α-subunit and locations of mutations associated with hyperkalemic periodic paralysis (HyperPP), paramyotonia congenita (PMC), and potassium-aggravated myotonia (PAM). (*Continued on facing page.*)

the gene that encodes the α1a subunit of the P/Q-type voltage-gated calcium channel, which is found within the locus (Ophoff et al 1996). Similar mutations have been observed in the homologous gene in tottering (*tg*) mice that display a form of absence epilepsy (Fletcher et al 1996).

Given the enormous number of different ion channels that are now known, we can confidently predict that many more channelopathies will soon be discovered.

GENETICS HAS ALSO CONTRIBUTED TO ELUCIDATING SOME FORMS OF THE MORE COMMON NEURODEGENERATIVE DISORDERS

Compared with the good progress that has been made in elucidating the genetic basis of the resulting changes of single-gene defects, progress in understanding the more common sporadic neurodegenerative disorders, such as amyotrophic lateral sclerosis (ALS) and Alzheimer's and Parkinson's disease, has been more modest. The common strategy for addressing these disorders has been to search for mutations in identified genes in the rare familial forms of the disorder, in the hope that this might provide some insight into the much more common sporadic cases of the disease. This latter endeavor has, however, proved frustrating.

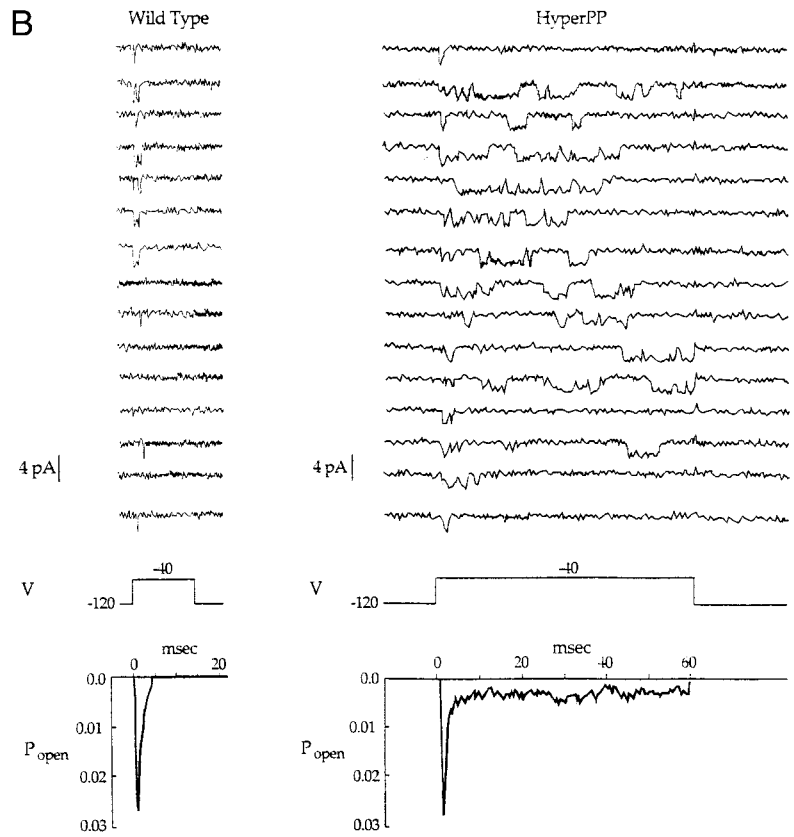

Figure 6 (*B*) Defective fast inactivation of Na channels in HyperPP (Met1592Val). (Tracings) Currents recorded from single Na channels in cultured human myotubes. In response to depolarization, both wild-type and mutant Na channels quickly open (pulse-like downward deflection) within a few milliseconds, indicating that activation was not impaired. In selected consecutive trials, failure of fast inactivation produced bursts of reopenings and prolonged open durations in channels with the Met1592Val mutation. Ensemble averages (below tracings) show that mutant channels inactivate for the majority of trials (rapid decline in P_{open}) but that intermittent bursts cause a small persistent current. pA, picoamps; V, volts; P_{open} probability. (From Cannon 1998.)

Amyotrophic Lateral Sclerosis

The hope that once the gene for the familial form of a disorder was identified it might lead fairly quickly to the discovery of the gene (or genes) responsible for the more common sporadic form of the illness was high when, in 1991, an extensive team of collaborating scientists reported the finding of a locus on the long arm of chromosome 21 that showed strong linkage to a familial form of ALS in

a group of 23 families (Siddique et al 1991). This initial finding was followed 2 years later with the discovery (by the same collaborating group) of mutations in the Cu/Zn superoxide dismutase (SOD 1) gene in 13 families with familial ALS (Rosen et al 1993).

ALS is characterized by a progressive generalized weakness and wasting of skeletal muscle that often leads to death, typically within 5 years of the onset of symptoms, although it can occur in as few as 10–12 months. It has long been known to be due to the progressive degeneration of spinal motoneurons and other large neurons in the cerebral cortex and brainstem. Approximately 10% of cases are inherited as autosomal dominant, with especially high penetrance after the sixth decade. Because clinically and pathologically the familial form of ALS (FALS) resembles the more common sporadic form of the disorder (although the familial form generally occurs at an earlier age), there was some hope that the identification of several different missense mutations in the SOD 1 gene in FALS might also lead to the discovery of the genetic basis of sporadically occurring ALS. Unfortunately, this has not proved to be the case, and indeed, it is now clear that SOD 1 mutations account for only a small proportion (~15%) of cases with FALS and other genes are certainly implicated (for example, see Chance et al 1998).

Currently, almost 72 different SOD 1 mutations have been reported in FALS, and several have been mapped onto the three-dimensional structure of the enzyme. Although it still is not clear how these mutations result in a gain-of-function phenotype (for discussion, see Siddique et al 1996), insights into these mutations have been gained from transgenic mouse models of ALS. Overexpression of certain SOD mutations in mice leads to muscular weakness and paralysis, and there is a fairly close relationship between the degree of overexpression of the mutated gene and the age of onset of motoneuron death. Unfortunately, the interpretation of these findings remains equivocal. Is the motoneuron death due to the accumulation of free radicals? Is it due to the emergence of a new enzymatic activity such as the formation of nitronium ion from peroxynitrite? Or is it due to the accumulation of some other toxic factor that is especially deleterious to motoneurons? Only further research will resolve these questions and, perhaps of even greater importance, lead to the discovery of the underlying cause of other forms of FALS and of the much larger number of cases of sporadic ALS.

Parkinson's Disease

Parkinson's disease (PD), which affects about 1 million people in the United States, is a late-onset neurodegenerative disorder for which there is increasing evidence for a substantial genetic contribution (for review, see Olanow & Tatton 1999). Patients suffering from PD develop increasing muscular rigidity, slowness of movement, a characteristic resting tremor, and disturbances of gait. The primary cause of the disorder is the progressive loss of dopaminergic neurons in the pars compacta of the substantia nigra accompanied by the appearance of intra-

cytoplasmic inclusions known as Lewy bodies. As the disorder progresses, neurodegenerative changes are seen in other parts of the brain, including the cranial nerve nuclei, the nucleus basalis, and parts of the hypothalamus. Approximately 5–10% of PD patients have a clear family history, suggestive of an autosomal dominant pattern of inheritance. Monozygotic twins who develop the illness before the age of 50 show a high degree of concordance, and the overall incidence of the disorder is appreciably higher in individuals who have one or more affected relatives.

A number of candidate genes have come under scrutiny as possibly being associated with PD, including those for *ApoE-4, tyrosine hydroxylase, superoxide dismutase 1* and *2, glutathione peroxidase,* and those that encode various dopamine receptors. To date, none of these has been found to be consistently affected, although a number of patients with familial PD have been found to have point mutations affecting certain components of the respiratory chain commonly referred to as complex 1 (a group of 41 subunits, including 7 encoded by mitochondrial genes); however, the absence of disease-specific mutations or a maternal inheritance pattern indicative of a mitochondrial gene involvement has cast doubt on the significance of these findings. At present, the best evidence for specific mutations is in the gene encoding the protein α-synuclein in the 21–23 region of the long arm of chromosome 4 and in the gene encoding parkin on the long arm of chromosome 6.

The locus on the long arm of chromosome 4 was first identified in a large Italian-American kindred in which several members had developed PD at a relatively early age. At autopsy, the brains of the affected individuals showed most of the pathological changes characteristic of Parkinson's disease, including the presence of Lewy bodies. Similar mutations have been identified subsequently in several unrelated Greek families (Polymeropoulos et al 1997). About 85% of the affected individuals studied had a single G to A base change in the *α-synuclein* gene, resulting in an alanine to threonine change in amino acid 53 of the protein. In a German family, a different base change resulting in an alanine to proline transition at amino acid 30 has been reported (Krüger et al 1998). These findings are of special interest because α-synuclein is an abundant component of Lewy bodies, and the reported mutations have been suggested to cause misfolding of the protein, rendering it susceptible to aggregation and, hence, less accessible to degradation by the ubiquitin proteasomes (Nussbaum & Polymeropoulos 1997).

Convincing though these findings appear, mutations in the α-synuclein gene probably represent a relatively rare cause of the disease. The French Parkinson's Disease Genetics Study Group has recently reported an analysis of the entire coding sequence of the α-synuclein gene in 25 families (24 French and 1 Italian) with dominant Parkinsonism (French Parkinson's Dis. Genet. Stud. Group 1998). In none of the index cases was there evidence of either mutations or polymorphisms in the coding region of the gene. And in a separate unpublished study (see French Parkinson's Dis. Genet. Stud. Group 1998), the Ala 53 Thr mutation was not found in 230 cases of familial Parkinsonism. On this basis, the authors

conclude that unless mutations are present in noncoding regions of the α-synuclein gene, it can, at best, be only a minor locus for PD in the populations they have examined.

More recently, a Japanese group reported finding mutations in a different gene in patients with autosomal recessive juvenile Parkinsonism (AR-JP) located on the long arm of chromosome 6. Within this critical region they have identified an open reading frame that encodes a protein of 465 amino acids that they have termed parkin. Parkin is widely expressed but apparently enriched within brain tissue, including the substantia nigra. It has some similarity to ubiquitin at its N terminus and has a zinc finger at its C terminus. In five patients with AR-JP studied by this group, the fourth exon of the gene was missing. Despite the absence of Lewy bodies in AR-JP, it is thought that parkin may act by interfering with ubiquitin-mediated proteolysis. The importance of mutations in the parkin gene in the etiology of AR-JP has been confirmed by the European Consortium on Genetic Susceptibility in Parkinson's disease, which has analyzed 35, mostly European, families. Several different point mutations were identified in the parkin gene, and in one case a deletion of exon 4 was found (Abbas et al 1999).

Finally, a susceptibility locus for autosomal dominant PD in patients from the United States, Italy, and Germany, at chromosome 2p13, has been identified. Included in this region are the genes for transforming growth factor-α and a neutral amino acid transporter that may be implicated in the pathogenesis of PD.

We have listed these various findings to indicate that a number of different single-gene defects probably can give rise to the familial forms of PD. We have even less of an understanding about the underlying cause of the more common sporadic form of the disorder. Furthermore, the relatively low concordance rates of PD in monozygotic twins makes it clear that nongenetic factors must play a significant role in the pathogenesis of PD. Again, many such factors have been considered, but with the notable exception of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity, none stands out as crucial in most cases of the disorder (Olanow & Tatton 1999).

Alzheimer's Disease

While the identification of the genetic basis of most degenerative neurological disorders has been frustratingly slow, appreciable progress has been made in the past decade in the understanding of some of the genes involved in Alzheimer's disease (AD). In part, this has been driven by the growing awareness—as life expectancy in developed countries increases—of the seriousness of AD and of the personal, social, and health care costs associated with the disorder. Although AD was first thought to affect a relatively small number of individuals in late midlife, it is now clear that it is the most common cause of dementia in the elderly, affecting perhaps as many of 40% of those over the age of 85 and accounting for about 100,000 deaths in the United States each year.

Clinically, the disorder begins with subtle changes in cognition and personality, with memory loss as one of its most striking early features. Pathologically, it is characterized by substantial loss of neurons in the cerebral cortex, hippocampus, amygdala, and elsewhere (including, most notably, the nucleus basalis, the principal source of cholinergic input to the cortex) and by two other distinctive changes—the appearance of large numbers of neuritic extracellular plaques and neurofibrillary tangles. The primary cause of the cell death is not known, but the neuritic plaques have been found to be composed largely of a 42/43–amino acid peptide called β-amyloid (Aβ), whereas the neurofibrillary tangles are formed of bundles of paired helical filaments of the microtubule-associated protein tau. Both sporadic and familial forms of the disease occur, the former accounting for by far the larger proportion of cases.

Genetic studies have identified three genes associated with familial AD: the gene encoding the β-amyloid precursor protein (APP), presenilin 1, and presenilin 2. In addition, there is considerable evidence that one isoform of apolipoprotein (APO) E (APO E4) is a significant risk factor for late-onset AD, acting as a dose-dependent age-of-onset modifier, and it is possible that two other isoforms, APO E3 and E2, may be protective (Strittmatter & Roses 1996). Almost certainly other genes are involved, as indicated by a recent whole-genome screen for late-onset familial AD, which identified a locus on chromosome 12 that was strongly linked to AD, and three other loci on chromosomes 4, 6, and 20, respectively, may also be involved (Pericak-Vance et al 1997).

β-Amyloid Precursor Protein To date most attention has been paid to the APP gene, on chromosome 21. This attention is perhaps not surprising, because the amyloid-containing plaques are such a striking feature of AD—and the first to be identified. The full-length cDNA of APP encodes a protein of just over 700 amino acids that has the Aβ peptide sequence near its C terminus. The APP sequence contains as many as 19 exons, and several splice variants have been found in the central nervous system and elsewhere (for review, see Schellenberg 1995). All pathogenic mutations associated with AD are found within or immediately adjacent to the Aβ sequence. This sequence is cleaved from the rest of the APP protein between residues 671 and 672 by a proteolytic cleavage referred to as the β secretase process, followed by a second (γ secretase) cleavage somewhere C terminus to residue 712 that results in a long (42 amino acid) and a shorter (40 amino acid) Aβ peptide (Haass & Selkoe 1993). Five different mutations in the APP gene have been associated with AD alone; a sixth has been found to cause AD in some patients but is more commonly associated with cerebral hemorrhage and angiopathy. The mutations that cause "pure" AD all result in the longer Aβ (1–42) peptide, which is the most abundant Aβ species in the neuritic plaques. From a mechanistic point of view, the APP mutations that cause familial AD appear to exert their affects by increasing the production of Aβ 1–42, probably by interfering with the normal processing and trafficking of the protein. As shown

below, disturbances in the normal processing of APP may represent a common feature in various genetic forms of the disease.

Several attempts have been made to replicate the pathological findings in AD using transgenes in mice (for review, see Price & Sisodia 1998). In all of those that led to Aβ deposits, the mutant APP transgenes were expressed at high levels in the central nervous system, and the brains showed dystrophic neuronal processes and typical plaque-like deposits of amyloid in the cerebral cortex, hippocampus, and amygdala. In one line of mice, the animals showed normal learning and memory in appropriate tasks at 3 months of age, but by 9–10 months they were clearly impaired in the same tasks (Price & Sisodia 1998). These and other mouse models hold considerable promise for our understanding of the pathogenesis of AD.

The Presenilins The gene for presenilin 1 (PS 1) was identified using a positional cloning strategy that led to the identification of missense mutations in a gene on chromosome 14, which encodes a 43-kDa protein (Sherrington et al 1995). Presenilin 2 (PS 2) was identified on chromosome 1 on the basis of its homology with PS 1; it encodes a 50-kDa protein. The sequence of both proteins predicts the existence of six to nine transmembrane helices that share more than 60% amino acid identity. So far, more than 35 mutations, mainly involving the conserved transmembrane domains or a region adjacent to a large intracytoplasmic loop, have been identified. All but one are missense mutations that appear to have a toxic gain-of-function effect (Hardy 1997). The evidence for this view is threefold: First, plasma from patients with PS mutations contain increased amounts of the longer form of the Aβ peptide; second, the brains of patients with PS mutations have abundant deposits of Aβ42, and cultured fibroblasts from these patients produce large amounts of Aβ42; and third, transgenic mice overexpressing mutant human PS genes show an increase in the Aβ42:40 ratio at 2–3 months of age, and by 10–12 months there are large numbers of amyloid deposits in their brains (Price & Sisodia 1998). Mutations in the presenilin genes are thought to account for about 20–25% of all cases of familial AD.

The function of the presenilins has been clarified unexpectedly by the finding in the nematode *C. elegans* of a protein (Sel-12) with considerable homology to the presenilins (Levitan & Greenwald 1995). Sel-12 has been shown to be involved in the notch-delta signaling pathway that is important in cell-fate determination. Because mutations in Sel-12 can be rescued by the introduction of normal human PS 1 or PS 2, it seems likely that the presenilins serve the same signaling function in humans. A critical step in notch signaling involves cleavage of the protein and the translocation of the intracellular domain to the nucleus. The finding that null mutations in the *Drosophila* presenilin gene abolish notch signaling and nuclear translocation (Struhl & Greenwald 1999) serves to emphasize this point. The deletion of PS 1 in mice greatly reduces γ secretase activity and increases the deposition of the Aβ peptide. This finding and the existence of two

conserved aspartates in the presenilin protein has led Selkoe and his colleagues to suggest that PS 1 may either be the long-sought-for γ secretase or a unique diaspartyl cofactor for γ secretase (Wolfe et al 1999).

APO E4 The common isoforms of APO E are encoded by three APO alleles, E2, E3, and E4, which differ from one another as a result of amino acid substitutions at two specific codons. The APO E4 allele is associated with an increased risk of AD in about 50% of all cases of late-onset AD and shows a clear dose-dependent relationship: Individuals with two copies of the allele have an earlier onset of symptoms than those with only one copy, and the latter have an earlier onset than those who lack E4 alleles. The amount of Aβ42 deposited in the brain is also correlated with the number of E4 alleles. By contrast, the E2 and E3 alleles are protective. APO E3, but not E4, binds to tau; preventing its aggregation may well be related to this difference (for review, see Lendon et al 1997).

The discovery of an increased frequency of the APO E4 allele in late-onset cases of AD (compared with age-matched controls) is of considerable importance because of its occurrence in both familial and sporadic forms of AD. For reasons that are not yet understood, APO E4 appears to also influence the age of onset of AD in individuals with mutations in APP but not in patients with mutations in either of the presenilins. Whether this argues for a difference in the pathogenesis of the disorder in the two groups of patients (even though both are associated with an increase in the Aβ42:40 ratio) remains to be determined. It is important to note, however, that not every individual with the E4 allele will develop AD, so although APO E4 screening is a useful adjunct to diagnosis, it cannot be used as a reliable predictor of AD in any given case.

The biological basis for the association of the E4 allele and AD remains to be determined, but it is significant that APO E is found in senile plaques and is associated with neurofibrillary tangles; and there is also some evidence that the three different isoforms differ in their affinity for Aβ. Finally, lipoproteins associated with APO E4 are cleared from blood more efficiently than are those associated with the other two isoforms, and within the brain, APO E4 is now known to be synthesized by astrocytes.

Despite the significant progress that has been made in our understanding of the genetics of AD, much remains to be done. In particular, it is not yet established that the deposition of Aβ is the primary etiological factor in the disorder, and it seems likely that many other factors (including probably several unknown environmental factors) contribute to its onset and progression.

PSYCHIATRIC DISEASES ILLUSTRATE THE DIFFICULTIES IN ANALYZING COMPLEX, POLYGENIC DISORDERS

Schizophrenia

The dramatic insights provided by molecular genetics into the underlying causes of so many neurological disorders have prompted a resurgence of interest in the genetics of mental illness. Surprisingly, the first evidence that genes are important in the susceptibility to schizophrenia was provided as early as 1938, by Franz Kallman, who was impressed with the fact that the incidence of schizophrenia throughout the world is uniformly about 1%, even though the social and environmental factors vary dramatically. He also found that the incidence of schizophrenia among parents, children, and siblings of patients with the disease is 15%, which is further evidence of a genetic component of the disease.

To distinguish genetic from environmental factors, Kallman (and subsequently others) turned to twin studies comparing the rates of illness in identical (monozygotic) and fraternal (dizygotic) twins. These studies have established that the concordance rate for schizophrenia in monozygotic twins is about 45–50%, compared with only about 5–15% in dizygotic twins—about the same as for other siblings. In fact, an individual's risk for developing schizophrenia increases dramatically with the degree of relation to an affected person (Gottesman 1991). The risk for anyone in the general population developing schizophrenia is about 1%. If one's first cousin has schizophrenia, the risk doubles to 2%; the risk for siblings of a person with schizophrenia is 9%; if a sibling and one of the parents has the disease, the risk rises to 16%. Taken together, these data suggest a strong genetic contribution to the susceptibility to schizophrenia. However, the fact that identical twins are discordant at least 50% of the time indicates that genetic factors are necessary but not sufficient to account for the relatively high frequency of the disorder. Multiple causality is also evident from studies of the transmission of the disease within affected families. Routine studies of pedigrees are usually sufficient to pinpoint whether a disease is due to mutation in a single gene and is transmitted in a dominant, recessive, or X-linked Mendelian pattern. It is clear from essentially all the studies that have been carried out that the transmission of schizophrenia does not follow this simple pattern. Although the penetrance of even a dominant trait, such as HD, is age dependent, it nevertheless approaches 100%, and there are only minor nongenetic factors influencing the final phenotype. With recessive genes, such as phenylketonuria, even if both parents carry the trait, neither may display the phenotype but one in four of their children will have the disease. Moreover, typical single-gene disorders are very rare: For example, HD and most other dominant diseases have a prevalence of about 1/10,000, whereas schizophrenia and manic depressive illness have a prevalence of 1 or 2/100.

There are several likely explanations for the unusual genetic transmission of schizophrenia. To begin with, all the available evidence points to schizophrenia

as a disease that can involve allelic variations at many loci. Moreover, in any single individual, it may require the concerted action of several of these alleles to express the disease (each allele contributing only a part—in some instances possibly only a very small part—to the final appearance of the disorder). Moreover, the genes involved are likely to exist in a variety of allelic forms with low penetrance (Karayiorgou & Gogos 1997). Finally, what we currently classify as schizophrenia may prove in time to be a number of different disorders, each involving a different, or conceivably overlapping, set of genes. Understanding schizophrenia, therefore, will require both an understanding of how genes combine to create a predisposition to a disease and how the environment influences that predisposition. Of course, the fact that generally many genes are involved does not exclude the possibility that in rare individual cases, or in a few families, individual genes may play a major role in the susceptibility to the disease.

In recent years, the study of schizophrenia has turned increasingly to systematic searches of the entire genome (for recent reviews, see Karayiorgou et al 1996, Karayiorgou & Gogos 1997, Andreasen 1999). Although no specific gene has yet been found, the initial searches have uncovered three loci that may be associated with schizophrenia. One locus is on the long arm of chromosome 22 (22q); another is on the short arm of chromosome 8 (8p), and the third is on the short arm of chromosome 6 (6p). In each case, the locus has been narrowed to a region containing as many as 50–100 genes. From a neurobiological point of view, the region on 8p is of particular interest because it overlaps with a region thought to be involved in agenesis of the corpus callosum (Dobyns 1996). This overlap suggests the intriguing possibility that this region of chromosome 8 might encode a number of genes concerned with neuronal connectivity. In fact, Swayze et al (1990) report that about 0.2% of patients with schizophrenia have at least a partial agenesis of the corpus callosum compared with 0.07% to 0.05% in the population at large. This indicates an enrichment for this disorder in patients with schizophrenia of up to fourfold.

As these arguments make clear, the analysis of schizophrenia poses an extraordinary challenge to a molecular, cellular, and genetic analysis of disease states (Karayiorgou & Gogos 1997, Andreasen 1999). So far, there are no reliable biological markers, no good insights into the pattern of inheritance, or into the neuropathology of the disease. The challenge remains to identify several interacting alleles, each of which may be only moderately contributory but which together cooperate to interfere with mental processes as complex as social interactions and thinking.

Manic Depressive (Bipolar) Disorder

The evidence for genetic factors in manic depressive illness (MDI) or bipolar disorder is even more striking than that for schizophrenia, but the search for the genes involved has been almost as frustrating, in part for the same reasons: the

difficulty of diagnosis in all but the most typical cases, the polygenic nature of the disorder, and a disturbing history of mistaken claims.

MDI was first described by Kraepelin (1921) as running "its course in isolated attacks more or less sharply defined from each other. . . . Accordingly," he added, "we distinguish manic states [characterized by] flight of ideas, exalted mood, and pressure of activity, and melancholia or depressive states with sad or anxious moodiness and sluggishness of thought and action." But complicating the diagnosis, he went on to say, " . . . we observe also clinical 'mixed forms' in which the phenomena of mania and melancholia are combined. . . . " (see MacKinnon et al 1997).

MDI is said to affect about 0.5–1% of the US population, but it is likely—if variant forms of the disorder are considered—that the incidence may be appreciably greater. MDI is found more frequently in individuals with one or more relatives who have suffered from the disorder than in the population at large or than in individuals with relatives who have had other psychiatric illnesses. The concordance rate among monozygotic twins is variously reported as 50–70% but may be considerably higher among those where suicide has occurred (Rifkin & Gurling 1991). Among dizygotic twins, the concordance rate is between 15 and 30%. In keeping with these findings, it is known that the concordance is significantly higher between adopted children and their biological parents than between affected children and their adoptive parents.

It is hardly necessary to recount the many reported linkage studies carried out—sometimes on large, closely knit groups—that have not stood the test of later reexamination (for recent review, see MacKinnon et al 1997). Suffice it to say that in the past 5 years, convincing evidence for the existence of a distinct locus on the short arm of chromosome 18 has been presented and independently confirmed (Berrettini et al 1994). McMahon et al (1995) have also found evidence for yet another locus on the long arm of chromosome 18, which, it is interesting to note, shows evidence of being paternally inherited. Unfortunately, the region defined at both these loci is large—as much as 80 centimorgans—and likely to contain scores of genes that might be associated with MDI. It is likely also that other genes, for example, those for monoamine oxidase and tyrosine hydroxylase, could plausibly be linked to MDI (at least in some patients) and may yet be found to contribute to the disorder. The rapid progress that is currently being made in the human genome project gives hope that within the foreseeable future, mutations in specific genes will be identified and their relative contributions to the underlying pathology of MDI clearly established.

THE PREFRONTAL CORTEX, WORKING MEMORY, AND SCHIZOPHRENIA

A major weakness in current approaches to schizophrenia and bipolar disorders is the lack of a well-established neuropathology. Recently there has been a resurgence of interest in the possibility that the prefrontal cortex is disordered in

schizophrenia. The prefrontal cortex is part of a larger neural circuit involved with attention, memory, orderly thinking, and planning cognitive functions that are disturbed in schizophrenia. Because the prefrontal cortex has become a focus for current studies in cognitive neuroscience, independent of schizophrenia, we complete our review by considering some of the salient findings in the normal and disordered functioning of this region.

Interest in the prefrontal cortex dates to 1848 when Harlow (1848) described the now famous case of the railroad foreman named Phineas Gage. An accidental explosion drove a tamping iron through Gage's prefrontal cortex, a finding recently confirmed by computer-generated reconstructions of the lesion from the entry and exit wounds of the tamping iron in Gage's preserved skull (Damasio et al 1994). Gage survived the incident, but his personality was changed. Before the accident he was reliable and industrious; afterward he was unreliable, drank a great deal, and eventually became a homeless drifter. Subsequent studies of patients with similar lesions and of nonhuman primates with lesions of the prefrontal cortex confirm that the prefrontal cortex, particularly the dorsolateral area, plays a critical role in judgment and in long-term planning. Patients with damage to the prefrontal association areas tend to achieve little in life, and their behavior suggests that their ability to plan and organize everyday activities is diminished. Nevertheless, they continue to show good general intelligence; and their perception and long-term memory remain reasonably intact.

In the 1930s, Carlyle Jacobsen at Yale turned to nonhuman primates to study the function of the prefrontal association areas in the planning of actions and provided the first experimental evidence that one key function of the prefrontal cortex is in the sequencing of behaviors over time (Jacobsen 1935, Jacobsen & Nissen 1937). This led Jacobsen to propose that the prefrontal cortex is involved in short-term memory. Later research has suggested that prefrontal lesions do not produce a generalized deficit in short-term memory but rather they produce a specific deficit in working memory, a component of short-term memory used for the temporary storage and the temporal integration of information that guides future actions. Working memory is the means whereby a person is thought to retain knowledge of the information provided by an environmental cue in order to perform the appropriate behavioral response after the cue has been removed.

The idea of a working memory was introduced in 1974 by the cognitive psychologist Alan Baddeley (Baddeley & Hitch 1974), who suggested that many apparently simple aspects of everyday life—carrying on a conversation, adding a list of numbers, driving a car—depend on a short-term memory mechanism that he called working memory. This memory, Baddeley argued, integrates moment-to-moment perceptions across time, rehearses them, and combines them with simultaneous access to archival information about past experience, actions, or knowledge. According to Baddeley's view, working memory has three distinct components: First, there is an articulatory loop for verbal memories, including memories for language; second, there is a parallel component, a sketch pad for visual memories and action; and third, there is a component that functions as a

central executive, coordinating the flow of attention between the articulatory loop and the sketch pad. This model has given rise to the idea most clearly developed by Joaquin Fuster (1997) and Patricia Goldman-Rakic (1992) that at least aspects of working memory are represented in component regions of the association areas of the prefrontal cortex.

Aspects of Working Memory Can Be Studied on the Cellular Level in the Prefrontal Cortex

Of the several areas that comprise the prefrontal cortex, the cortex surrounding the principal sulcus has been studied in greatest detail. In monkeys, even a relatively small lesion to this area produces a deficit in working memory (Figure 7). The idea that this part of the cortex is involved in working memory has been further supported in monkeys by cellular neurophysiological studies. Fuster & Alexander (1971) first recorded the activity of neurons in the cortex surrounding the principal sulcus and discovered that these neurons respond only to stimuli at a particular position in the visual field, usually in the contralateral hemifield, and only during tasks requiring directed eye or limb movements toward that site. A given neuron begins firing when the visual stimulus is presented, and it continues to fire during any delay period in the task (even after the stimulus is turned off), when the monkey is presumably maintaining a working memory of a spatial location in anticipation of directing movement toward it. If, on a given trial, a prefrontal neuron stops firing before the response is required, it will fail on that trial, which suggests that the monkey has "forgotten" the spatial location (for review, see Fuster 1997). More recently, Goldman-Rakic (1992) has extended these studies and confirmed that continuous activity in prefrontal neurons underlies the behavioral continuity required to execute the task. Even when the visual cue is no longer there, the prefrontal neurons continue to fire throughout the delay period, and they do so for only restricted regions of visual space. Thus, Goldman-Rakic has argued that neurons in the principal sulcus have memory fields that are analogous to visual receptive fields (Goldman-Rakic 1992, 1994); they have the ability to use stored knowledge about a particular part of the external world and to guide appropriate motor responses at a later time based upon that knowledge.

Cognitive activity in prefrontal cortex is not restricted to cases in which the response is directed to the spatial location of a remembered stimulus. Prefrontal neurons are also activated by tasks that involve indirect stimulus-response mappings, for example when a movement is made away from the remembered location, or the location is inferred based on nonspatial information (Funahashi et al 1993, Kim & Shadlen 1999, Ferrera et al 1999). These observations support a role for prefrontal cortex in the decision-making component of working memory.

The Prefrontal Cortex and the Search for a Neuropathology of Schizophrenia

Schizophrenia is characterized most prominently by disorders of thought, attention, and executive function. Because the prefrontal cortex is thought to be involved in attention and in the planning and execution of complex behaviors,

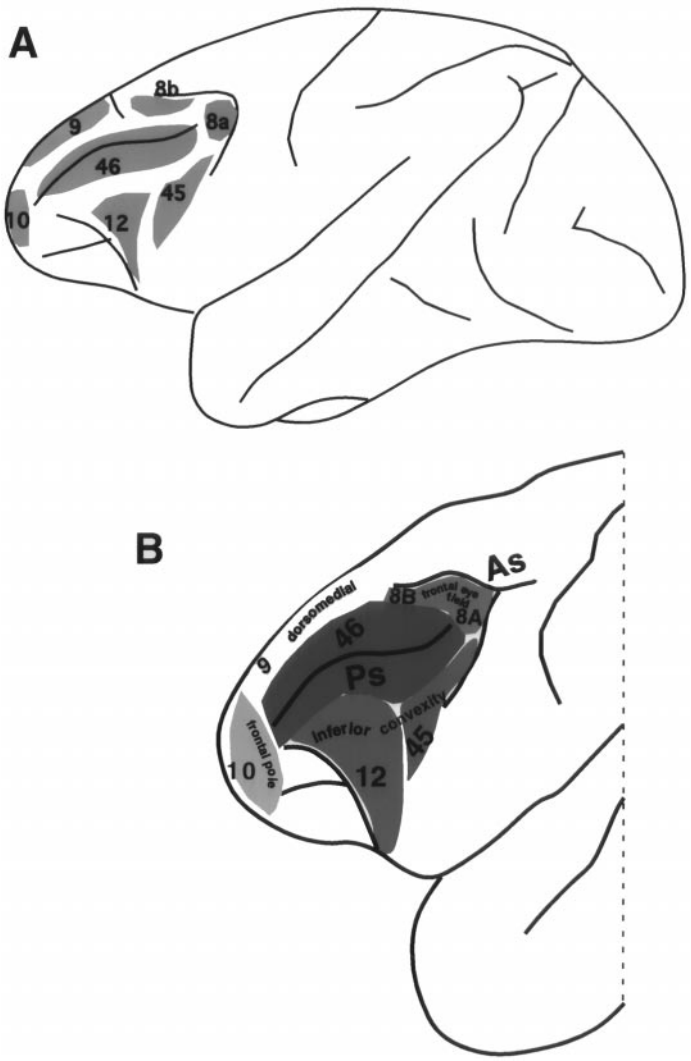

Figure 7 Diagram of rhesus monkey brain revealing the basic divisions of the prefrontal areas, based on mapping system of Walker (1940). (Shading) The principal sulcus (PS) (area 46) and other areas on the dorsolateral surface are shown. The prefrontal cortex has been divided into a number of cytoarchitectonic areas on its ventral and medial surfaces, but these are not shown. AS, arcuate sulcus. (From Goldman-Rakic 1994.)

volitional functions that are thought to be specifically disordered in schizophrenia, it has long been thought that schizophrenia is likely to reflect disordered functioning in association cortices and particularly the prefrontal cortex. This idea has been supported repeatedly by imaging studies of the brains of schizophrenic

patients, which have consistently revealed hypofunction of the prefrontal cortex. Thus, when challenged by a task that engages prefrontal functions (such as the Wisconsin Card Sorting Test), blood flow into the prefrontal areas of schizophrenic subjects increases much less than that seen in normal subjects (for review, see Weinberger et al 1992).

Indeed, patients with schizophrenia are cognitively impaired on the Wisconsin Card Sorting Task (Goldberg et al 1987), and they also are impaired on spatial working memory tasks (Park & Holzman 1992). Moreover, 40–50% of first-degree relatives of schizophrenic patients display this same cognitive deficit even though they lack clinical symptoms. By contrast, patients with bipolar disorder showed no such impairment (Park & Holzman 1992, Park et al 1995a,b).

Schizophrenic patients also show abnormalities in voluntary eye movements: saccades and smooth-pursuit movements, as well as oculomotor or delayed-response tasks (Park & Holzman 1992, Sereno & Holzman 1995). Electrical stimulation studies have identified a region of prefrontal cortex in the anterior bank of the arcuate sulcus, the frontal eye fields, from which saccadic eye movements can be evoked with low currents (Bruce et al 1985). Indeed, recently, a subregion of the frontal eye fields has been identified where stimulation selectively produces smooth-pursuit eye movement rather than saccades (Gottlieb et al 1993).

Because some symptoms of schizophrenia respond to blockade of D_2 dopaminergic receptors, schizophrenia has been thought to involve dopaminergic dysregulation. The prefrontal area of humans and other primates has a particularly prominent dopaminergic innervation. Local depletion of dopamine from this area produces effects that are similar to lesions of the dorsal prefrontal cortex. Thus, Goldman-Rakic and her colleagues have found that performance of working-memory tasks (such as delayed-response tasks) is disrupted in monkeys when dopamine is depleted from the cortex surrounding the principal sulcus (by means of a localized injection of the drug 6-hydroxydopamine, which selectively destroys dopaminergic terminals) (see Brozoski et al 1979).

To see whether these abnormalities in working memory, in eye movements, and in other voluntary movements were associated with any anatomical alteration of prefrontal cortex, Selemon et al (1995, 1998) examined the brains of 16 patients with schizophrenia and 19 normal subjects using three-dimensional counting methods. In the brains of schizophrenic patients they found a 17% increase in neuronal density in prefrontal cortex (area 9) and a 10% increase in the striate cortex (area 17). Based on cell counts, they conclude that the number of neurons per se does not differ between schizophrenic and normal subjects. Rather, they postulate that the increased neuronal density could result from loss of surrounding neuropil (dendrites, dendritic spines, and presynaptic terminals) and the consequent shrinkage of interneuronal space. This finding is interesting in view of the extensive pruning of synaptic connections that normally occurs in the prefrontal cortex in humans and in monkeys during adolescence (Huttenlocher 1979, Bourgeois et al 1994). In monkeys, for example, the density of dendritic spines on

layer 3 pyramidal neurons declines by 50% between 2 and 4 years of age, the period of adolescence for this species. These considerations have given rise to the idea (Feinberg 1982, Hoffman & Dobscha 1989) that schizophrenia may result from an abnormality that leads to excessive pruning. According to this view, excessive pruning could result in a loss of dendritic neuropil, and this could contribute to the dilatation of the lateral ventricle seen in schizophrenia. Indeed, the most common finding in patients with schizophrenia is an enlargement of the lateral and third ventricles with a concomitant widening of the sulci, especially in the frontal lobe, reflecting a reduction of cortical tissue (Andreasen et al 1982). Other analyses, however, suggest a more general cortical disturbance in schizophrenia. For example, the medial temporal lobe is sometimes thinner and the outer portion of the hippocampus is smaller than normal, especially on the left side.

Because the ventricular abnormalities seem the most reliable of the anatomical abnormalities, the question has arisen as to the nature of the relationship between the ventricular abnormalities and the genetic predisposition for schizophrenia. This question has been examined in a study of monozygotic twin pairs of which only one twin had schizophrenia (Suddath et al 1990). In 12 out of 15 such twins examined, the twin with schizophrenia had larger ventricles compared to the normal twin. The twin with schizophrenia almost invariably also had a smaller hippocampus on the left side, and this reduction in hippocampal mass correlated strongly with the reduction in blood flow in the left prefrontal cortex. These imaging studies of structural and functional anatomy suggest that the hippocampus may be part of a cognitive system, together with the prefrontal cortex, that is impaired in schizophrenia.

The fact that twins who share identical genes have anatomical differences in their brains suggests that genes alone do not account for these structural changes. Rather, the structural changes result from the interaction of genes and other factors that may include developmental abnormalities, perinatal injury, or postnatal pathology. Thus, these anatomical studies suggest that the development of schizophrenia may be a two-step process in which genetic predisposition is necessary but not sufficient to produce the disease (Weinberger et al 1992).

Unfortunately, the past history of research on the anatomical basis of schizophrenia does not encourage one to think that this disease has a single anatomical locus, much as the genetics does not lead one to think that it results from one unique pattern of expression. It therefore seems likely that the prefrontal abnormalities and other newly discovered anatomic abnormalities in cortical development (see Pakkenberg 1989, 1992) represent only a current sampling of what may well prove to be several basic and contributory anatomical defects. Nevertheless, the recently described anatomical lesions do share several interesting characteristics (Bogerts 1993, Heckers 1997). First, the abnormalities are already present at the onset of the disease and generally do not progress thereafter. Second, some of the disturbance in cytoarchitecture reflect neurons that are misplaced and disorganized, which is suggestive of a developmental abnormality. Third, there is

an absence of gliosis, a reaction that usually accompanies infectious, inflammatory, or degenerative neurological processes. Finally, people who become schizophrenic have often shown delayed neurological development during their infancy and childhood.

CONCLUSION

The convergence of the various subfields concerned with studying brain and behavior to the common discipline of neuroscience has greatly enhanced our understanding of both the biology of the brain and its control of behavior. Moreover, this synthesis has helped demystify the brain for the rest of the biological community and thereby has attracted the interest of many young biologists to the problems posed by the brain and by behavior.

What has been the impact of this new synthesis for neurology and psychiatry? The contributions of neuroscience to psychiatry and neurology fall into two broad categories: conceptual and experimental. From a conceptual point of view, neuroscience is beginning to create an intellectual framework for exploring mental function, a framework encompassing both psychiatry and neurology. As a consequence, one can foresee a possible realignment and merger of the two clinical disciplines along lines that might provide a more coherent approach to a variety of disorders of cognitive functioning, including autism, mental retardation, AD, and age-related memory loss, in which both disciplines have a historical interest.

Experimentally, neuroscience has provided genetic and cell biological insights into the causes and pathogenesis of a number of major neurological illnesses, such as muscular dystrophy, HD and the channelopathies, and familial forms of AD and ALS. These monogenic disorders have lent themselves readily to current genetic approaches to human disease. By contrast, neuroscience has not, as yet, succeeded in making important progress in the much more difficult area of psychiatric disorders. This difference can be traced to two reasons. First, the genetic diseases of neurology that have yielded most readily to molecular analysis have been monogenic diseases. By contrast, genetic analyses have not yet reached the point where they are effective for the analysis of the polygenic diseases that characterize most psychiatric disorders, such as schizophrenia, depression, bipolar disorder, and anxiety states. As a result, the finding that the E4 allele of APO E is a risk factor for nonfamilial AD is of great interest, because this is perhaps the first finding of a gene contributing to a polygenic disease of the brain.

Second, even prior to the advent of molecular biology, neurology justifiably prided itself on its ability to localize lesions to specific sites not only in muscle, peripheral nerve, and the spinal cord but also within the brain. As a result, the modern era of molecular neurology was based on a solid foundation of anatomically well-characterized and diagnostically valuable neuropathology. Unfortunately, we do not as yet have this foundation for psychiatric disorders. Until recently, thinking about the biological foundations of psychiatric disorders could only be guided by psychopharmacology, by the response of patients to psycho-

pharmacological agents that are helpful in the treatment of depression and schizophrenia. In no case so far has any major psychiatric disease been localized to particular disturbance of a functional system in the brain. It is for this reason that the recent suggestion that the prefrontal cortex may be involved in schizophrenia and that the amygdala may be critical for anxiety disorder is so welcome.

In fact, of all the major mental illnesses, there is at the moment perhaps the greatest expectation for an anatomical understanding in the case of anxiety states. The realization that the amygdala is critical for the expression of fear (for review, see LeDoux 1996) has encouraged the hope that this insight will lead to a beginning understanding of anxiety states—a family of disorders that includes simple phobias, panic disorders, chronic anxiety, and obsessive-compulsive disease. Because fear can be studied successfully in mice, it opens up the possibility of animal models of disease; unfortunately, such models are not as easily developed for schizophrenia and bipolar disease.

For their part, however, neurology and psychiatry have provided neuroscience with a broad array of fascinating clinical conditions with disturbances in perception, in motor ability, and in specific cognitive functions, which will continue to challenge us well into the twenty-first century. To meet this challenge we will need to do more than study patients with specific disorders. We will need the benefit of genetically tractable model organisms, such as worms, flies, and mice, not only to discover the genes of interest but also to understand the function of the proteins they encode and to learn how identifiable mutations can lead to recognizably disordered clinical phenotypes.

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