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Prospectus

Epilepsy and Antiepileptic Drugs: A Speculative Synthesis

Eugene Roberts

The solution to the various problems involved in normal and abnormal function of the nervous system will require, minimally, an integrated understanding of the wiring diagrams (neuroanatomy and ultrastructure), the electrical and chemical characteristics of the conducting units and transmitting mechanisms in the circuits (neurophysiology and neurochemistry), the interactions between the fast neural systems and the relatively slow endocrine mechanisms (neuroendocrinology), the behavior of the organisms whose activities are regulated by them (psychology), and a thorough understanding of the mechanisms of the actions of drugs (pharmacology). An attempt must be made to broaden the interface between the several areas of study and to assimilate their basic principles.

Epileptiform phenomena are observed naturally and can be produced experimentally in virtually every region of the vertebrate CNS, even in isolated fragments thereof. The sheer diversity of the phenomenology is overwhelming. In the case of human beings, behavioral manifestations, including various types of motor and psychomotor seizures, intense pain, learning difficulties, and schizophrenia-like dementias, have been found to be associated

with electrographically detected abnormalities. The production of seizures may be associated with a variety of genetic and environmental factors, among the latter being developmental defects, physical injury to neural tissue, circulatory disturbances, fever, viral and bacterial infections, autoimmune reactions, toxic effects of various chemical agents, prolonged bouts of hypoxia or hyperoxia, neoplasia, dietary deficiencies (e.g., vitamin B₆), periods of extraordinarily heavy inputs to particular neuronal regions, and innate supersensitivities to particular sensory inputs. Seizures are a final common path taken by nervous tissue when excitatory activity occurring within it exceeds the capacity of the tissue to modulate the activity.

An attempt will be made in this chapter to find some unities amidst a bewildering diversity and to bring current observations and suggestions into sufficient focus so that future work in the development of antiepileptic agents and related treatments will be facilitated. Although many patients with convulsive disorders have been helped greatly, an adequate rationale for the design of treatments and the devisal of new drugs still has not been developed. One major difficulty is that a myr-

iad of interrelated events takes place between changes in activities of individual neurons and the occurrence of seizures. Effective drugs and treatments that prevent seizures from occurring may intervene at one or more neural processes. There are many ways by which normal activity of a given neuron or neural sector can be increased. The chief mode may be by increasing neural inputs. But increases in the amounts of substances (e.g., catecholamines, peptidic or steroid hormones) that may release rate-limiting intracellular chemical reactions, increased availability of limiting extracellularly furnished constituents, and/or the exit of inhibitory substances as a result of vasodilation and vascular permeabilization, enhancement of conductile properties of neurons, elevations in core temperature, and decreases in the efficacy of the neural inhibitory apparatus may also importantly come into play. All of the latter factors are, at least to some extent, understandable, measurable, and manipulable. Less understood are the events that underlie the occasional escape of individual neurons into maladaptive individual spiking activity as the system begins to show "overheating." However, the phenomenology underlying the sudden and discontinuous appearance of hypersynchronous, paroxysmal discharges of groups of neurons seen in seizures still remains *terra incognita*. Neuroscientists understand this no better than social scientists comprehend the transition that occurs when a gathering of excited individuals becomes a raging, destructive mob. In general, the techniques currently available allow only narrow windows through which to observe an awesome multidimensional and kinetic panorama.

We are fortunate in that there are a number of substances known to be reliably effective in controlling different types of seizures, as well as a variety of other substances that can produce seizures. If we can identify the effects of these substances that are most germane to the control or production of seizures, they may serve as lanterns to light the way to a better understanding of nervous system function, as well as to more adequate methods of treating

and preventing seizure disorders. On the other hand, an understanding of the mechanisms of action of antiepileptic substances may be no more revealing than knowledge of the effectiveness of a bandage in controlling bleeding from a scratch is in revealing the underlying disorder in clotting in hemophilia. In our effort to seek answers, we must deal with problems of circuits, membranes, molecules, and time and be able to sketch in freely wherever lacunae exist in our factual knowledge. Although many hundreds of papers have been consulted in writing this chapter, relatively few citations have been included in order to avoid extensive redundancy with the preceding chapters.

WHAT ARE NEURONAL CIRCUITS LIKE IN PRINCIPLE?

All neurons can be thought of as possessing an innate capacity for firing spontaneously. Thus, if a particular neuron were isolated from its biologic context and maintained under suitable environmental circumstances, it would exhibit a characteristic firing pattern close to its maximal potential rate, which would be paced by inward ionic currents and their inactivating processes. For each neuron, this pattern would be a unique result of the interaction of its genetic potential with the environmental influences that had acted on it up to the moment of observation. Each neuron would "speak with its own voice," since multiple environmental gradients exist from the time of earliest development; hence no two neurons in an organism, although similar, could be identical in every respect. Differences could probably be detected with sufficiently subtle methods (morphologic, physiologic, biochemical, immunologic) even in adjacent neurons subserving similar functions and originally arising from the same embryonic area or belonging to the same clone.

Most neurons in their normal environment in intact organisms are members of neuronal circuits and have largely ceded their autonomy while becoming citizens of an integrated neuronal community. Many neurons do not fire

spontaneously at their maximal rates in their normal settings; some do not fire spontaneously at all. Glial cells may serve as one major restraining influence on spontaneous activity of neurons by removing substances from the extraneuronal environment in the regions of synapses, by adding substances to it, or by preventing diffusion of substances liberated from neurons in such a way as to shunt depolarizing ionic currents, thereby decreasing intrinsic excitatory levels of neuronal membranes below their spontaneous firing levels. Another type of inhibitory influence is exerted by the effects of neurotransmitters liberated onto neurons from inhibitory neurons. Such inhibitory neurons could be only phasically active, with their activity depending on the inputs to them; or they could be tonically active, spontaneously firing cells. Also, some inhibitory neurons might release inhibitory transmitter constantly without an action potential, the rate of release being determined by the degree of membrane polarization (21). By opening appropriate ionic channels or releasing ion carriers, inhibitory transmitters would act essentially as chemical voltage clamps on their postsynaptic sites by allowing shunting ionic currents to occur and, in the presence of suitably set ionic pumps, hyperpolarization of excitable membranes to take place. Release from inhibition could be achieved by direct depolarization of the inhibited cell, by inhibition of the inhibitory neurons, or most commonly by a combination of both. Most neuroscientists who were trained before the early 1950s had been conditioned by past training to consider neuronal circuits almost entirely in terms of excitatory events. The first neurons in the circuits were presumed to be excited by some input and to pass on excitatory or depolarizing messages synaptically in such a way that there would result a progressive excitation passed from neuron to neuron, until the final neuron in the circuit would depolarize an effector cell, muscle, or gland. Inhibition was considered to play, at most, a vague modulatory role. Only within the last 20 years have the major and essential roles of neural inhibition and

disinhibition in nervous system function become fully recognized.

It has not proven particularly useful to view seizures as events occurring at one end of a continuum of normal neural events. Observations in the literature and our intuitions suggest that a discontinuity exists between normal neural function, local and global, and the explosive all-or-none discharges characteristic of seizure states, much in the way that a discontinuity exists between reactions that precede the explosion of a nuclear device and the explosion itself. As indicated above, a myriad of factors, few of which in intact animals currently seem to be quantitatively determinable, may contribute to the vectors that eventually allow neural tissue to attain the seizure state. It seems likely that a useful strategy in our discussion might be to assign relative weights to some of the known factors and to attempt to deal only with a few of the major ones, until the data force us to begin to include the others. Therefore, it would be useful to give a brief account of the current state of our knowledge of the locations of neurons that release gamma-aminobutyric acid (GABA), the major inhibitory component in the vertebrate central nervous system, and some of the roles they play in neural information processing. There now is much evidence to indicate that interference with the function of these neurons can, among other defects in neural information processing, lead to seizures. This concurs with the conclusion of Chapter 15, which deals with the mechanisms of action of convulsant drugs: "The main effect appears to be an influence to block neurotransmitter-activated increase in chloride conductance," a process that is often mediated by the release of GABA from GABAergic neurons.

GABA NEURONS IN THE VERTEBRATE CNS: A GENERAL STATEMENT

The demonstration that a particular synapse is a GABA-releasing one is not sufficient evidence to identify it unequivocally as an inhibitory synapse. Supporting physiologic evidence is necessary for the assignment of such a func-

tional role. However, in almost all instances adequately studied to date in vertebrate nervous systems, the overall effects of GABA, where GABA synapses have been proven to exist, have been found to be inhibitory either at presynaptic or postsynaptic sites. GABA typically produces an increase in membrane permeability to Cl^- that can be measured as an increase in membrane conductance. Therefore, one way that this naturally occurring transmitter can counteract the depolarizing action of excitatory processes is to maintain the polarization of a cell at an equilibrium level near that of its resting value, acting essentially as a chemical voltage clamp. GABA also has been shown to exert a hyperpolarizing inhibitory effect, because in many instances there exists an outwardly directed Cl^- pump. However, when there are relatively high intracellular Cl^- concentrations, possibly because of the presence of an inwardly directed Cl^- pump, GABA can produce a decrease in membrane potential, or depolarization, a mechanism possibly involved in presynaptic inhibition. It is possible that at some GABAergic synapses the Cl^- pump may not be operative and only the shunting effect takes place, whereas at others both shunting and hyperpolarization occur.

I cannot overemphasize the importance of detailed physiologic analyses, employing suitable intracellular recording techniques, in ascribing functional roles to GABA neurons in particular settings and in studies of GABA agonists, antagonists, and amplifiers. Although there is often a close relationship between the generator potential and frequency of firing, it is well known that a variety of chemical changes can produce a dissociation, or uncoupling, between the two. Therefore, mechanisms of actions of substances on membranes cannot be established definitively from experiments in which only their effects on firing rates of neurons are determined. The elucidation of molecular mechanisms of GABA action will require the use of all of the biochemical and biophysical techniques available for the study of the effects of substances on

membranes and probably even more knowledge than we possess today.

Much of the published work reviewed in this volume, as well as other reports from recent studies, suggests that convulsants such as picrotoxin, bicuculline, penicillin, and pentylenetetrazole and anticonvulsants such as diphenylhydantoin, diazepam, and phenobarbital, among other effects that they exert, may decrease or increase, respectively, the efficacy of GABA in producing the membrane effects described above. It already is clear that, where active, the above substances do not compete effectively with GABA at receptor recognition sites. Their action may be exerted at other membrane sites, perhaps on one or more of the several steps that intervene between GABA attachment to its receptor recognition sites, the consequent increase in chloride conductance that GABA is known to produce, and the inactivation of the conductance increase.

MEMBRANE DEPOLARIZATION AND RECOVERY: A BRIEF SKETCH OF TRANSMITTER ACTION AND INACTIVATION

When a neural membrane is depolarized by the impingement of an excitatory transmitter or by some other means, the relationship of calcium ions to membrane components is altered in such a way that, in addition to the inward flow of sodium, there probably is also a closely related inward calcium flux. Although the sodium current usually is responsible for most of the depolarization of the postsynaptic membrane, the increase of free intracellular calcium activates the opening of potassium channels. The outward potassium current then serves to repolarize the cell and, in many instances, to produce a hyperpolarization before the calcium balance is restored via the action of a $\text{Ca}^{2+}\text{-Mg}^{2+}$ ATPase, and the potassium channels are closed. The action of $\text{Na}^+\text{-K}^+$ ATPase restores the mono-cation balance. The increase in free intracellular calcium, brief as it may be, also is believed to

trigger the sequence of events that is important for the release of the metabolic reactions required for recovery from activity as well as for the possible retention at presynaptic and postsynaptic sites of a biochemical "memory" of the experience. During this period, various enzymes related to cyclic nucleotide metabolism, as well as phosphoprotein phosphatases and phosphokinases, are activated. As a result, there are alterations in degrees of phosphorylation of membrane components and enzymes, with resulting release of metabolic recovery reactions, alteration of membrane affinities for anions and cations, and, in general, occurrence of cascades of interdependent reactions throughout the cellular machinery. At any step in the early events, genetically determined or environmentally induced abnormalities could result in the occurrence of pathological processes. Inadequacies in the function of the $\text{Na}^+ \text{-K}^+$ ATPase could produce a chronically depolarized neuronal state, which might make a neuron hyperexcitable. Abnormalities in the $\text{Ca}^{2+} \text{-Mg}^{2+}$ ATPases also might make a neuron overly sensitive to depolarizing influences. Interestingly, it has been reported that Ca^{2+} -ATPase activities are lower in audiogenic seizure-prone DBA mice during the period of maximal seizure susceptibility than in the C57 and C3H strains, which are seizure-resistant (55).

The state of the membrane and the processes which are involved in its excitation and recovery phases are in constant interaction with all influences impinging on the membrane of a nerve cell. The problem in attempting to control neurons which themselves are hyperexcitable and which can recruit others into their pathologically hyperactive domains devolves on our ability to identify the sites at which the balance may be restored in such a manner as to prevent the occurrence of interictal and paroxysmal discharges. Currently our knowledge of the nature of the major excitatory transmitters in the CNS is meager, and meaningful pharmacologic approaches to their manipulation are virtually nonexistent. However, our knowledge of the properties of

cationic channels in excitable membranes is rather extensive. On the other hand, although considerable knowledge of the GABA system per se has been accumulated in the 28 years since its discovery, the state of our knowledge of the anion channels or carriers is primitive, to say the least.

The impingement of GABA, and possibly other inhibitory neurotransmitters, obviously is an important counterbalance to the depolarizing influences exerted on neuronal membranes by passive cation leakage and by impinging excitatory influences. A current major pharmacologic objective in many laboratories is to develop procedures and substances which will allow manipulation of various aspects of the GABA system *in vivo* in a rational and predictable manner. From what has been discussed previously, it is obvious that it would be of great importance from an experimental point of view, as well as in the development of therapies, to be able to specifically decrease or increase the effectiveness of GABA neurons at will, either in the CNS as a whole or regionally. There are minimally six ways by which it might be possible to influence the activity of the GABA system: by altering (a) the activity of the GABA neurons themselves, (b) the synthesis of GABA, (c) its release, (d) its postsynaptic effectiveness, (e) the synaptic inactivation of GABA by carrier-mediated transport, and (f) its metabolic destruction by transamination and the subsequent oxidation of the carbon chain. Enhancement of any of the first four of the above processes alone or together and/or decreases in carrier-mediated transport and catabolic processes presumably should lead to enhanced GABA function, whereas the converse would be expected to result in decreased effectiveness of this system.

There are potentially at least four independent entities in membranes that are involved in the postsynaptic operation of the GABA system: (a) a GABA recognition site, (b) an anion channel or anion carrier protein, (c) a GABA removal and transport mechanism, and (d) an anion pump. A variety of endogenously occurring membrane-located sub-

stances, protein and lipid and other types of molecules ranging from ions to hormones, as well as a host of exogenously administered substances could act as regulators of this assembly. It could be postulated that the most precise operation of such a multiunit system would depend on a noncovalent coupling of the potentially independent constituents at postsynaptic membrane sites. This would occur in such a manner that the impingement of a given number of GABA molecules released from presynaptic sites would be followed by configurational changes which would result in the inward movement of Cl^- , the extent of which would be determined largely by the time required for removal of GABA from its recognition sites in the receptor complex. GABA may be inactivated at synapses by a mechanism that involves binding to unique membrane recognition sites, different from those for the receptor-anionophore complex; and its subsequent transport out of the synaptic junction probably is by a Na^+ -requiring process that is similar to that for many other substances. Compounds that potently and specifically block the binding of GABA to the GABA recognition site of the inactivation system, which do not affect the transport part of the process and possess no mimetic or antagonistic properties at GABA receptor sites and which do not become false transmitters, might be effective amplifiers of GABA action at synapses at which GABA normally is liberated. It is likely that removal of GABA from its receptor recognition site would almost immediately inactivate the mechanisms involved in Cl^- movement. The closely associated Cl^- pump mechanism might begin its operation as soon as Cl^- activity in its vicinity is increased by a small given amount, and might act sufficiently rapidly to maintain the ionic gradient, so that hyperpolarizing inhibition again could take place when GABA molecules would be liberated onto postsynaptic sites.

All of the above postulated components may be composed of genetically independent subunits and also may have an existence sepa-

rate from each other in the fluid structure of neuronal membranes. The same anionophore and anion pump systems also might associate with glycine recognition sites, perhaps operating as part of the glycine inhibitory mechanism. The GABA recognition entity, at least in some invertebrate systems, might even associate with Na^+ and K^+ ionophores, as well as with the one for Cl^- (66). The GABA removal and transport system appears to be ubiquitously distributed in synaptic and non-synaptic portions of neurons and in glial cells and probably often may not be associated with the other components of the GABA apparatus. One of its functions could be to act as a fail-safe device to ensure that the effects of fortuitous accumulation of nonsynaptically liberated GABA or that resulting from an overactivity of GABA neurons would be minimized. This is necessary because it has been shown in a number of instances that GABA can effect increases in Cl^- conductance when applied (a) to nonsynaptic regions of neural membranes, (b) to neurons with no known inhibitory input, or (c) even to nonmyelinated axons (7).

The conditions at postsynaptic sites of operative GABA synapses may be such as to favor the appropriate association of the above components into tightly coordinated units, the degrees of coupling of the individual components being dependent to some extent on the liberation of transmitter or of some specific macromolecular constituents from presynaptic sites. In turn, the postsynaptic supramolecular association in membranes through some intracellular representations may help regulate the amounts and rates of production of the individual components, perhaps even at the transcriptional or translational levels. In this connection it is of interest that when the immunocytochemical technique was employed in the developing rat cerebellum, glutamate decarboxylase (GAD) was found to be present in growing neurites in close association with small vesicles prior to the time the neurites made protosynaptic contacts; differentiation of these contacts coincided with the se-

questration of GAD into synaptic terminals and predated the establishment of contacts between presynaptic and postsynaptic elements of developing synapses (34). One would like to know whether the same is true for GABA postsynaptic receptor-ionophore complexes or whether contact between GAD-containing nerve endings and the postsynaptic membranes must take place prior to the synthesis of the individual components and/or the organization of these complexes.

The task now becomes one of developing techniques at the molecular level for the study of the individual components of this system and the properties of their assemblies. The pharmacology of the assemblies need not be identical with that of the individual components nor with some simple additive properties.

TRANSMITTERS AND CYBERNENES

At this point it is advisable for conceptual purposes to distinguish between at least two major classes of neurons in the vertebrate central nervous system. It appears that some neurons, such as those that may release GABA, acetylcholine, glutamate, aspartate, and glycine, probably are involved in direct point-to-point information transmittal. In other words, release of transmitter from the presynaptic endings of such neurons affects postsynaptic sites in such a way that, within approximately a millisecond, excitatory or inhibitory information is transmitted to postsynaptic sites, recognized, and the signaling substance removed. There are other groups of neurons, such as those that release catecholamines, serotonin, a variety of peptides, prostaglandins, and possibly other substances, which current evidence suggests may act chiefly by liberating their characteristic substances more generally into whole regions that contain various neuronal elements as well as glial cells and blood vessels. These substances may exert relatively long-lasting effects on blood flow and capillary permeability as well as more direct metabolic and trophic effects

on the cellular elements, possibly setting the gain on the efficacy of individual synapses, on specific types of synapses, or on all of the synapses in a given region. Their effects may be exerted at many functional loci, often via the cyclic nucleotides, such as transmitter synthesis, the postsynaptic control of ion channel open time after transmitter impingement, and the setting of windows on which of the total range of potential firing frequencies may be employed by given neurons or groups of neurons. These effects could be exerted by a large variety of cascading molecular mechanisms.

Such neurons, as mentioned above, may furnish the "oil" required for the neuronal machinery to function smoothly. That is, the effects of the neurons releasing such substances may be analogous to that of squirting oil into inadequately lubricated, but intact machinery, the parts of which will not function properly if sufficient oil is not furnished or if they are over-oiled. However, the oil is not part of the actual machinery. We would like to suggest that in many instances in which they act in the CNS, the catecholamines, serotonin, neurally released peptides, and prostaglandins may serve to optimize regional nervous system activity in relation to functional demands without themselves necessarily being involved in specific information transmittal. It is proposed to call such substances, which influence neural function without themselves necessarily being involved in neurotransmission, cybernenes, according to the suggestion of Roger Guillemin. For example, immunocytochemical, isotope labeling, and physiologic experiments are compatible with the suggestion that norepinephrine neurons in rat brain largely perform global, hormone-like functions, although occasionally they also may participate in typical synaptic relationships (9).

An inappropriate balance between availability and distribution of such cybernenes and activity in neural circuits could result in gross malfunction of the CNS, such as may be found in Parkinson's and Huntington's diseases or schizophrenia. It is striking that when the substantia nigra is stimulated, physiologically re-

corded signals in the corpus straitum do not seem to be greatly altered when the nigrostriatal dopamine neurons are destroyed by 6-OH-dopamine or when the action of dopamine is blocked completely by large doses of haloperidol. Also, the spontaneous firing rates of cells in the caudate nucleus are not altered by dopamine-depleting lesions of the nigrostriatal pathway. This suggests that the physiologically relevant signals are carried by fibers of nondopaminergic nigral neurons and that the effects of dopamine released from the dopaminergic fibers are not informational in the strictest sense of the word, as suggested above. A phenomenon related to the above is described in many anecdotal accounts about parkinsonian patients, obviously suffering from a defective functioning of nigrostriatal dopaminergic neurons, who can fully mobilize normal and adaptive physical activity in an emergency but who relapse into the typically inactive parkinsonian state as soon as the emergency is over. The above and the therapeutic effects of exogenously supplied levodopa in parkinsonism are compatible with the suggestion that the neuronal "hard-wired" circuitry in the neostriatum is potentially available and that the dopaminergic neurons furnish dopamine, the "oil" required for the neuronal machinery to function smoothly. In contrast, it seems likely that relative overactivity of the dopamine and/or the noradrenergic systems in particular brain regions are associated with psychotic and schizophrenic disorders, and the favorable action of neuroleptic drugs and propranolol in some of these conditions probably is attributable, in part, to their dampening of the effects of these systems, possibly by receptor blockade.

From the preceding discussion it can be seen that the cybernene and neurotransmitter systems can be considered as entities having somewhat different, but complementary, functions in the CNS. They are mutually interactive in all instances and in all neural regions. The main idea is that when an organism perceives that a problem exists, the stimuli reflecting the problem release both through-put and

cybernenic circuits simultaneously, the latter helping maintain a general state of readiness at the outset. Once the nature of the problem is assessed in the CNS, internal decisions are reached with regard to which brain regions and what specific circuitry within these regions would be most likely to be employed in the solution of the problem. Then the cybernenic systems are focused onto elements of the pertinent neural machinery, liberating their chemical facilitators, as though sprayed from an oil gun, to ready specific neural components for appropriate action. This sets the stage for rapid recovery from the activity and for those plastic changes to take place which would make it possible for the organism to solve the same or similar problems more expeditiously in the future. This action of cybernenes would give the temporarily functionally favored regions priority on the available oxygen and nutrient supplies, would adjust the cyclic nucleotide levels so that appropriate neural and glial membrane sensitivities and permeabilities would be maintained; and the various intracellular enzymatic cascades would be adjusted to keep transmitter synthesis, release, and inactivation and general cell housekeeping reactions going at rates commensurate with the functional requirements. The cybernenes also actually might act in such a way as to decrease activities and shunt oxygen and nutrients away from those cerebral regions and circuits not germane to the solution of the particular problem at hand. There would be constant interactional adjustment between the through-put circuitry and the cybernene neurons, largely via inhibitory and disinhibitory actions of interneurons, so that the entire system, through gradually diminishing oscillations, would "home" in on the optimal functional state, the typical operation of a system with negative feedback. Disease states can occur when such a system goes out of the range in which cybernetic adjustment can take place.

The points at which structural and/or functional lesions can take place that would impair the cyberneticity of such a system are legion. It is, therefore, no surprise that every known

transmitter candidate and every potential cybernene under one circumstance or another has been thought to be involved in various types of neural dysfunction. Certainly this is the case in seizure disorders. In what follows, we will try to deal with seizures and the drugs that affect them in terms of the above ideas, while trying to identify those rate-limiting reactions or processes that the individual drugs may primarily affect.

Since a large body of evidence now suggests that, among their various roles, they serve an essential coordinative function within and between neurotransmitter and cybernene systems, it is logical, at this point, to focus our attention on GABA neurons once again.

IMMUNOCYTOCHEMICAL LOCALIZATION OF GABA NEURONS

Until quite recently, the localization of GABA neurons has been inferred by correlating microchemical, electrophysiologic, pharmacologic, and iontophoretic studies with what was known of the cytoarchitecture of specific regions of brain and spinal cord. A commonly used approach has been to study a particular biochemical variable in whole brain or in anatomically defined regions of brain or spinal cord. Analyses of GABA contents and GAD activities have been performed in almost all identifiable brain structures and in the spinal cord. Some studies have combined biochemical analyses with lesioning procedures in correlating specific neural degenerations with losses of L-glutamate decarboxylase, the enzyme that catalyzes the synthesis of GABA from glutamate. The distributions of the components of the GABA system also have been studied extensively by subcellular fractionation techniques in preparations from whole brain or selected regions. Interpretation of results from the above types of analyses at the cellular level often suffers from the lack of definition attributable to the presence of myriad cells of different types in any dissected region, and it is difficult to make specific conclusions about synaptic connectivi-

ties from them. Individual cell bodies of large neurons, such as Purkinje and Deiters' cells, and even portions thereof, have been dissected out and subjected to microanalyses. In the latter instances, it is not always possible to ensure absence of contaminating presynaptic endings from other neurons or of glial cell constituents, and, therefore, their contribution to a particular measured chemical variable is not always known. The technical difficulties involved in the latter type of work also usually preclude the study of large numbers of neurons in this manner.

The above approaches have not made possible a definitive understanding of how GABA neurons might participate in information processing in different parts of the vertebrate CNS. Direct localization of GABA neurons and nerve endings now has been achieved by immunocytochemical visualization of GAD. GABA neurons have been found to form axodendritic, axosomatic, axoaxonic, and dendrodendritic synapses in the various regions of the rat CNS studied to date, which include spinal cord, cerebellum, cortex, hippocampus, olfactory bulb, retina, substantia nigra, and striatum. Electron micrographs of various types of synaptic junctions formed by presynaptic terminals containing GAD showed that in almost all instances several, if not all, of these types of GABA synapses are found in close proximity to each other (Fig. 1). In the section that follows, some of the salient findings in the several regions of rat CNS studied to date will be mentioned.

Spinal Cord

Immediately after the transduction event in a particular sensory receptor, there is a surprising amount of information processing, consisting of a coordinated interplay of excitation and inhibition. Two general aspects of sensory inhibition have emerged. Units in receptor systems inhibit their nearest neighbors via interneurons (intrasystem), causing enhancement of contrast and sharpening of input signals; and inhibitory and disinhibitory influ-

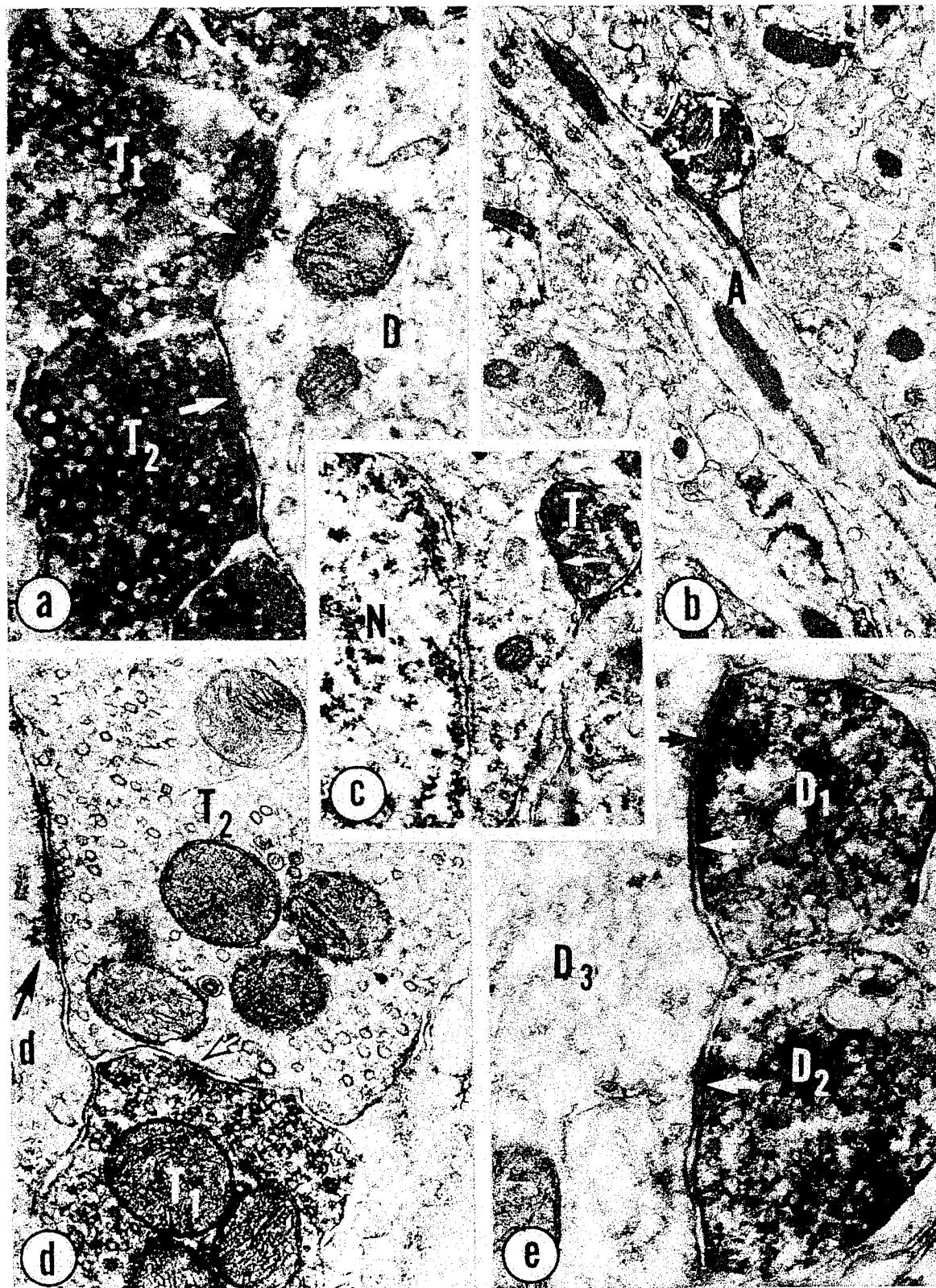


FIG. 1. Electron micrographs of various types of synaptic junctions formed by presynaptic terminals which contain glutamate decarboxylase (GAD), the enzyme that synthesizes the neurotransmitter γ -aminobutyric acid (GABA). All specimens were obtained from rat CNS. a: Axodendritic synapses in the substantia nigra. Two axon terminals (T_1 and T_2) filled with electron-opaque, GAD-positive reaction product are shown to synapse with a dendritic shaft (D) in the pars reticulata. One of the terminals (T_1) forms an asymmetric synaptic junction (arrow), while

ences from higher relay centers (intersystem) may impinge on the receptors to set their gain. Thus, even close to the place and time of entry of environmental information, the organism abstracts those aspects which are most essential for adaptation. Subsequently, the neural signals carrying the information from the sensory receptors enter the CNS. Many variable sources of excitatory sensory inputs are competing constantly for the attention of the organism. It would be asking a great deal of the first mainline central neural elements alone to adjudicate all of the competing claims. Nature has devised ways for helping resolve such conflicts. A great deal of information processing occurs even at the most peripheral levels of the CNS with the aid of networks of closely lying indigenous neurons, many of which were presumed to be GABA neurons. Our investigations of rat spinal cord have now confirmed the above presumption and have furnished a morphologic basis for the well-documented physiologic observations.

Light microscopic localization of GAD in the rat lumbosacral spinal cord showed heavy, punctate GAD-positive reaction product in the dorsal horn laminae I-III (1,33). Moderately heavy reaction product was also seen in the deeper dorsal horn laminae IV-VI, the medial aspect of the intermediate gray (lamina VII), and the region around the central canal (lamina X). A moderately light concentration of GAD-positive reaction product was observed in the ventral horn, and punctate deposits of reaction product also were seen on motoneuron cell bodies. The visually observed

punctate distribution of GAD-positive reaction product described above corresponded to GAD-positive synaptic terminals visualized by electron microscopy in comparable regions. Many more GAD-positive terminals were observed in dorsal horn laminae I-III than in the deeper laminae IV-VI. GAD-containing terminals in the dorsal horn were presynaptic to dendrites, cell bodies, and other axon terminals. The latter were more numerous in laminae II and III. GAD-positive synaptic terminals were presynaptic to large and small dendrites and motoneuron somata in the motor nuclei. In addition, small GAD-containing terminals also were presynaptic to larger axonal terminals, which were in turn presynaptic to motoneuron somata. The observation of GAD-positive terminals presynaptic to dendrites and cell bodies in both dorsal and ventral horns was compatible with the evidence suggesting that GABA terminals may mediate postsynaptic inhibition of spinal interneurons and of motoneurons.

Multiple dorsal rhizotomies were performed unilaterally at lumbar levels L1-L4 in adult rats, and the spinal cords were examined 24 to 48 hr later. Large numbers of degenerating terminals, probably entirely from cutaneous afferents, were observed in the ipsilateral substantia gelatinosa, but not contralaterally. However, the distribution of GAD-positive reaction product, most intense within laminae II and III, appeared to be normal on both sides of the cord. Electron microscopically, primary afferent terminals were found in various stages of degeneration on the

the other terminal (T_2) forms a symmetric synapse (arrow). $\times 33,000$. b: An axoaxonal synapse in the cerebral cortex. A GAD-positive axon terminal (T) is shown forming a symmetric synapse (arrow) with an axon initial segment identified by a dense undercoating of the axolemma (arrowheads) and a fasciculation of microtubules (e.g., asterisk). $\times 15,000$. c: An axosomatic synapse in the dorsal horn of the spinal cord. A probable synaptic junction (arrow) is shown between a GAD-positive terminal (T) and a neuron (N) in the substantia gelatinosa. $\times 19,300$. d: An axoaxonal synapse in the dorsal horn of the spinal cord. A synaptic junction (white arrow) is shown between the GAD-positive presynaptic terminal (T_1) and another synaptic terminal (T) which is not GAD-positive. In addition, T_2 is the presynaptic component of another synaptic junction (black arrow) with a dendrite (D). $\times 28,500$. e: Dendrodendritic synapses in the glomerular layer of the olfactory bulb. Two GAD-positive gemmules (D_1 , D_2) from dendrites of periglomerular neurons form synapses with a mitral/tufted dendritic shaft (D_3). One gemmule (D_1) appears to form a reciprocal synapse and the other gemmule (D_2) appears to be presynaptic only. $\times 40,500$. Directions of synaptic transmission are indicated by arrows in a-e. (Electron micrographs provided by R. P. Barber, B. J. McLaughlin, C. E. Ribak, and J. E. Vaughn.)

side of rhizotomies, and GAD-positive axon terminals were found to be presynaptic to degenerating primary afferent terminals in the substantia gelatinosa. The data furnish a chemomorphologic basis for the conclusion that the presynaptic inhibition of primary afferents is mediated by axoaxonal synapses formed between GABA-releasing interneurons and primary sensory neurons. A detailed analysis of the various synaptic relationships of GAD-positive terminals in the dorsal horn of the rat spinal cord has led to reasonable hypotheses about how release of GABA from these terminals could participate in such presynaptically modulated phenomena as primary afferent depolarization, the dorsal root reflex, and primary afferent hyperpolarization. A sche-

matization of some of the relations of GABAergic neurons in the substantia gelatinosa is shown in Fig. 2.

In a number of the synaptic complexes observed, a GAD-positive terminal was presynaptic to a primary afferent terminal, and in addition both kinds of terminals (i.e., GAD-positive and primary afferent terminals) were presynaptic to the same dendritic profile (Fig. 2D). In these cases, the same GABAergic axon terminal could mediate both presynaptic and postsynaptic inhibition. Depolarization of a primary afferent axon terminal by GABA could mean that a subsequent primary afferent volley would result in less transmitter being released. This reduced amount of released primary afferent transmitter would also have to

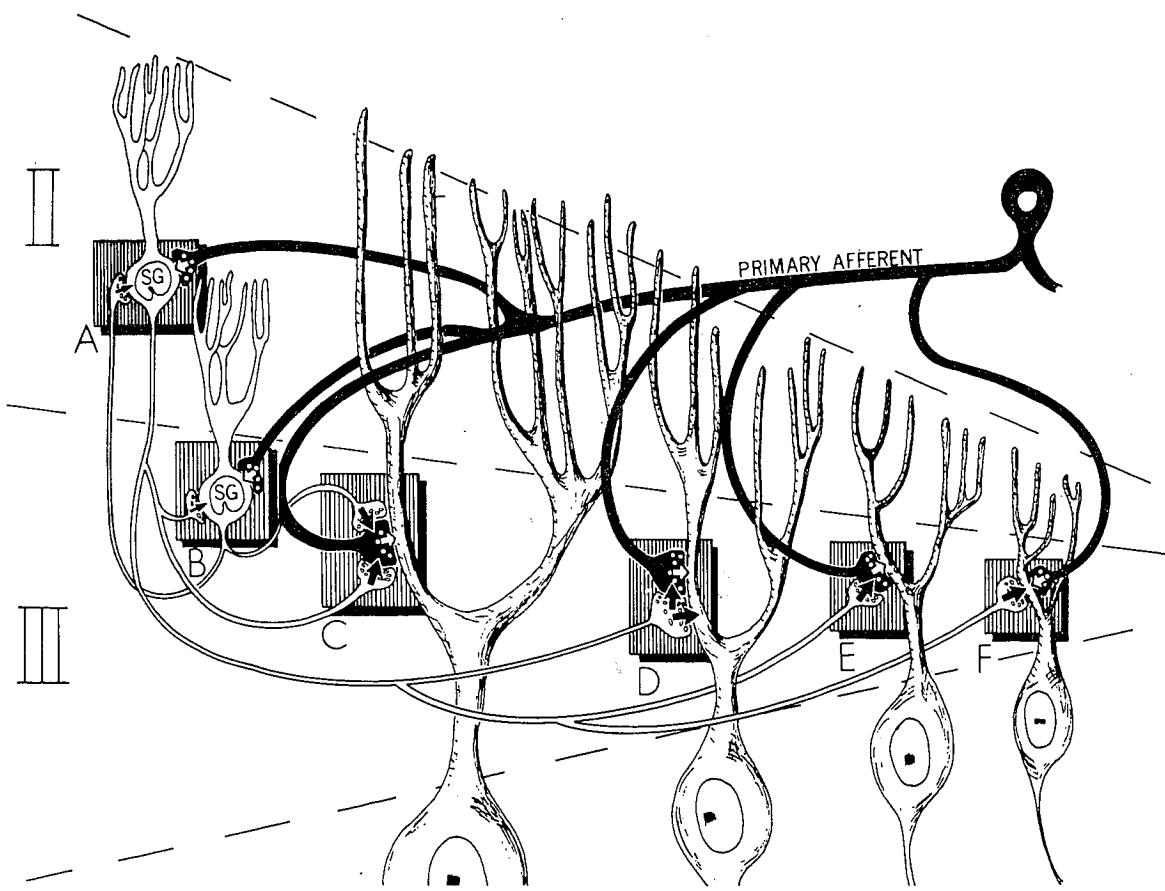


FIG. 2. Schematic representation of possible synaptic pathways within the substantia gelatinosa (Rexed's laminae II and III) which involve both GABAergic and primary afferent axon terminals. GABAergic substantia gelatinosa neurons (SG) are white, and a primary afferent neuron is black. The single primary afferent neuron represents all of the different neurons of this class whose axons terminate in the substantia gelatinosa. *Black arrows* indicate the direction of GABAergic synaptic transmission, and *white arrows* indicate the direction of primary afferent synaptic transmission. See text for discussion. (From Barber et al., ref. 1.)

exert its effect on a postsynaptic dendrite that had been hyperpolarized by the action of a GABAergic axodendritic synapse. Consequently, there would be a further reduction of the effectiveness of the primary afferent transmitter substance in producing an excitatory postsynaptic potential in the second-order neuron. It is plausible to assume that inhibitory synaptic complexes such as those established by the GABAergic terminals which make synaptic contacts with both presynaptic and postsynaptic elements (Fig. 2D) and those established by several GABAergic presynaptic terminals with the same primary afferent terminal (Fig. 2C) could form the center of the inhibitory gradient which has been described surrounding primary afferent fibers immediately following an afferent volley. The scheme illustrating possible synaptic pathways involved in a GABAergic modification of primary afferent activity (Fig. 2) is largely based on data accrued from investigation of rat lumbar spinal cord. However, primary afferent depolarization has been shown to occur at several spinal levels as well as in the cuneate nucleus, and it appears to be mediated by GABA in several species. Therefore, primary afferent depolarization mediated by the synaptic transmitter GABA would appear to be a rather general mechanism for the regulation of transmitter release from primary afferent terminals. On this basis, it is reasonable to suggest that synaptic pathways similar to those illustrated in Fig. 2 may be generally distributed throughout the substantia gelatinosa. Thus such pathways may provide repetitive units in which GABAergic interneurons presynaptically modulate the various exteroceptive impulses carried to the substantia gelatinosa by primary afferent fibers.

Cerebellum

The cerebellum was a favorable site for investigation of possible substances which may mediate the activity of neurons with inhibitory functions because more extensive correlative neuroanatomic and neurophysiologic analyses

have been made of the cerebellum than of any other structure in the vertebrate brain. The overall function of the cerebellar cortex is probably entirely inhibitory. The Purkinje cells, the only output cells of the cerebellar cortex, inhibit monosynaptically in Deiters' and intracerebellar nuclei. The basket, stellate, and Golgi II cells, which lie in the cerebellar cortex, are believed to play inhibitory roles within the cerebellum. The basket cells make numerous powerful inhibitory synapses on the somata, axon hillocks, and initial axon segments of the Purkinje cells. The superficial stellate cells form inhibitory synapses on the dendrites of Purkinje cells. The Golgi II cells make inhibitory synapses on the dendrites of granule cells. Afferent excitatory inputs reach the cerebellum via the climbing and mossy fibers, which excite the dendrites of the Purkinje and granule cells, respectively. The latter are believed to be the only cells with an excitatory function that lie entirely within the cerebellum.

Even the first comprehensive biochemical laminar analyses of the GABA system suggested the possibility that all of the inhibitory cells of the cerebellum (Purkinje, basket, stellate, and Golgi) might use GABA as transmitter. Subsequently, evidence was adduced for the occurrence of both GAD and GABA in high concentrations in the deep cerebellar nuclei where the Purkinje cell axons terminate (65). Electrophysiologic and pharmacologic studies strongly suggested that Purkinje cell terminals might liberate GABA as transmitter. Application of the immunoperoxidase technique at the light level showed an intense punctate deposition of reaction product around the Purkinje cells (Fig. 3c), suggesting the impingement of many nerve terminals containing GAD on these neuronal surfaces. At the electron microscopic level, GAD appeared to be highly localized in certain synaptic terminals in close association with the membranes of synaptic vesicles and mitochondria but not within these organelles. GAD-positive terminals, presumably largely from Purkinje cells, were seen on the somata and proximal

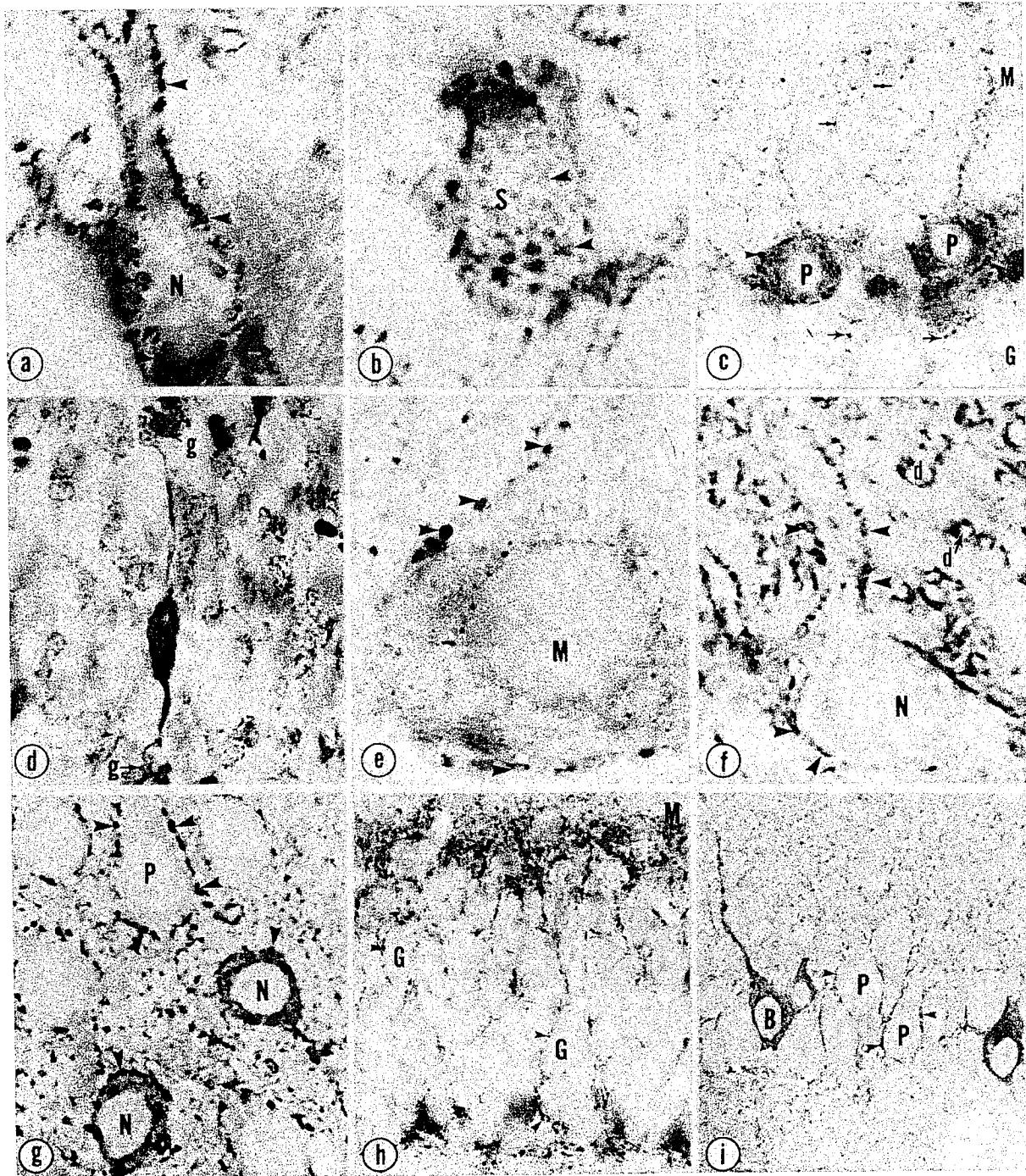


FIG. 3. GAD-positive terminals in rat central nervous system. **a:** Deep cerebellar nucleus (nucleus interpositus). A neuron (N) is shown studded by round, GAD-positive terminals (arrowheads), which are probably Purkinje cell axonal terminals ($\times 1,113$). **b:** Deep cerebellar nucleus (nucleus interpositus). A grazed neuronal soma (S) showing numerous GAD-positive terminals (arrowheads) on the somal surface ($\times 1,113$). **c:** Cerebellar cortex. The Purkinje cell somata are studded with GAD-positive terminals (arrowheads), which may be basket cell terminals. In the molecular layer (M), there are numerous GAD-positive punctate structures (arrows), and in the granular layer (G), GAD-positive punctate structures (Golgi II axonal terminals) are associated with glomeruli ($\times 462$). **d:** Granular layer of the cerebellum from tissue treated with colchicine to block axon flow. The Golgi II neuronal soma (N) has GAD-positive cytoplasm, and two of its processes are seen to terminate on glomeruli (g) ($\times 430$). **e:** Ventral horn of the spinal cord. A single motoneuron (M) exhibits several GAD-positive terminals (arrowheads) on the somal membranes ($\times 1,100$). **f:** Globus pallidus. A neuronal profile (N) is shown which has numerous GAD-positive terminals (arrowheads) studding its surface. Several dendritic cross-sectional profiles (d) also are covered by punctate,

dendrites of neurons in the deep cerebellar nuclei (Fig. 3a and 3b). Similarly, terminals presumably arising from basket, stellate, and Golgi type II cells also were strongly positive for the enzyme. Thus all of the cerebellar cortical cells known to be inhibitory were shown probably to use GABA as transmitter. Direct injections of colchicine into the cerebellar cortex, disrupting axoplasmic transport of GAD and other proteins, made it possible to demonstrate that the proximal dendrites and somata of Purkinje and Golgi type II neurons and the somata of basket and stellate cells contained detectable accumulations of GAD-positive reaction product (46). The GAD-positive reaction product was concentrated around the cisternae of the Golgi apparatus and was not seen in nuclei or nucleoli. The Golgi apparatus may be a way station for newly synthesized GAD prior to axoplasmic transport to pre-synaptic terminals.

Olfactory Bulb

Morphologic and physiologic aspects of the mammalian olfactory bulb have been studied extensively. In addition, high levels of GAD and GABA were found in the external plexiform, glomerular, and granule cell layers of this laminar structure. The mitral and tufted cells, which receive impulses from the olfactory nerves and from extrabulbar sources, are inhibited via reciprocal dendrodendritic synapses on their dendrites in the external plexiform and glomerular layers formed with the indigenous granule and periglomerular cells. Pharmacologic studies suggested that both of the latter interneuronal cell types are GABAergic. GAD has now been localized

in the olfactory bulb by immunocytochemical methods at the light and electron microscopic levels (48). Light microscopic studies demonstrated GAD-positive puncta throughout all layers of the olfactory bulb with the greatest concentration in the external plexiform layer and in the glomeruli of the glomerular layer. The cytoplasm of many neuronal somata in the granule and glomerular cell layers was GAD-positive, but not the cytoplasm of mitral and tufted cell somata. The GAD-positive staining of presumed granule and periglomerular neuronal somata also extended into their dendrites for many microns. There is evidence that some of the latter also may be dopaminergic.

Electron microscopic observations confirmed the presence of GAD-positive reaction product within the cytoplasm of granule and periglomerular neurons. Also, in the external plexiform layer, reaction product filled many of the granule cell gemmules which form reciprocal dendrodendritic synapses with mitral cell dendrites. The presence of GAD within granule and periglomerular cells strongly supports suggestions based on physiologic and pharmacologic studies that these inhibitory interneurons use GABA as their neurotransmitter.

Basal Ganglia

The nigrostriatal-pallidal system is concerned to a considerable extent with processing information related to proprioceptive, vestibular, and visual stimuli in the service of coordinating mechanisms involved in the physical orientation of an organism in its perceived space-time continuum. The caudate nu-

GAD-positive terminals ($\times 1,102$). g: Visual cortex from tissue treated with colchicine. Two neurons (N) have GAD-positive product in their cytoplasm while pyramidal cells (P) in layer V have clear cytoplasm. There are some GAD-positive terminals (arrowheads) on the same somal surface of all of the neurons shown here ($\times 658$). h: Dentate gyrus (Ammon's horn). Numerous GAD-positive terminals (arrowheads) stud the profiles of granule cells (G). In the molecular layer (M), there is a heavier investment of GAD-positive terminals around apical dendrites of the granule cells ($\times 441$). i: Ammon's horn, treated with colchicine. A basket cell soma (B) exhibits cytoplasmic GAD-positive product, while pyramidal cell somata (P) do not. GAD-positive punctate structures (arrowheads), presumably basket cell terminals, cover the pyramidal cell somal profiles ($\times 392$). (I am indebted to my colleagues Robert Barber and Charles Ribak for the preparation of this figure.)

cleus, putamen, and substantia nigra exchange fibers with each other, as do the substantia nigra and globus pallidus. The globus pallidus and substantia nigra receive inputs from the caudate and putamen and appear to have two-way communication with the subthalamic nucleus. There also are thalamic, cortical, and midbrain inputs to the caudate and putamen. Most of the final results of the computations in the basal ganglia are sent via a fiber system from the globus pallidus to the nuclei of the thalamus and thence largely to the motor cortex. However, there are some nigrothalamic connections as well. In addition, there probably are connections between the globus pallidus and the midbrain tegmentum through which descending influences may be mediated. In this regard, it is of considerable interest that facilitation of the gamma motor neurons can be achieved by stimulation of a midbrain region close to termination of the fibers from the globus pallidus, as well as by stimulation of the caudate nucleus. Normal relations within and between the above structures must involve minimally a coordinated functioning of different groups of intra- and intersystem neurons whose transmitters and/or cytochromes may be GABA, acetylcholine, dopamine, serotonin, norepinephrine, and possibly still unidentified excitatory transmitters, one of which may be the polypeptide, substance P.

Large amounts of GAD-positive reaction products, seen throughout the substantia nigra in light microscopic preparations, appeared to be localized in punctate structures that were apposed to dendrites and somata. Electron microscopic studies revealed that most of the axon terminals in the substantia nigra were filled with GAD-positive reaction product and formed both axodendritic and axosomatic synapses (47). Many dendrites were extensively surrounded by GAD-positive terminals, which most commonly formed symmetric synaptic junctions, although on the same dendrites some also formed asymmetric synaptic junctions. The results were consistent with biochemical, pharmacologic, and physiologic

data which previously had indicated that neurons of the neostriatum and globus pallidus could exert a GABA-mediated postsynaptic inhibition on the neurons of the substantia nigra. The GAD-positive somata in the globus pallidus were medium-sized neurons. In the striatum, the medium-sized spiny neurons, with round or fusiform shapes, appeared to be GABAergic (44). The pattern of GAD-positive terminals associated with dendrites of pallidal neurons resembled that observed in the substantia nigra. The results from the latter study are consistent with the interpretation of the results of other investigations that have indicated that the striatopallidal and striatopeduncular pathways, as well as striatal local circuit neurons and/or collaterals from striatal projection neurons, use GABA as a neurotransmitter.

Cortex

GAD was observed in somata, proximal dendrites, and axon terminals of nonpyramidal neurons in the rat visual cortex in all cortical layers, including the immediately subjacent white matter (43; FIG. 3g). GAD-positive terminals formed symmetric synaptic junctions most commonly with dendritic shafts and somata of pyramidal and stellate neurons, and less frequently with dendritic spines and with initial axon segments of pyramidal neurons. Extensive pericellular plexuses were formed by these terminals with the somata of pyramidal neurons in layers III and V. Detailed morphologic study of the GAD-positive neurons indicates that they are aspinous and sparsely spinous stellate interneurons with extensive intracortical axonal arborizations. At the light level, GAD-positive somata were seen to have GAD-positive terminals on their surfaces. The localization of GAD within these neurons in combination with physiologic and pharmacologic data indicate that these local circuit neurons mediate GABAergic inhibition and disinhibition in the neocortex and probably play a key role in information processing.

Retina

Dense aggregations of GAD-positive puncta appear to form four separate sublayers within the inner plexiform layer of rat retina, a broad sublayer being adjacent to both the inner nuclear and ganglion cell layers, and two thin sublayers lying in the middle portion of the inner nuclear layer. GAD-positive staining was not observed in any of the other retinal layers. Electron microscopy showed GABAergic terminals from amacrine cells ending on bipolar and amacrine cell processes and on ganglion cell dendrites and somata (63). GAD-positive terminals were also postsynaptic to bipolar cells and occasionally were observed to form reciprocal synapses with bipolar terminals. It appears from the results that some classes of amacrine cells are the only GABAergic neurons in rat retina. It will be important to determine whether other transmitters, which are believed by some to be dopamine, glycine, and taurine, are found in some amacrine cells and what their relations are to the GABAergic amacrices.

Hippocampus

Some of the GAD-positive puncta appeared in light microscopy as pericellular baskets around pyramidal and granule cell somata, and these puncta corresponded to the known distribution of basket cell axon terminals (46; Figs. 3h and 3i). GAD-positive reaction product also was observed in somata and dendrites. GAD-positive reaction product filled the somata and bipolar dendrites of many horizontal neurons located near the boundary between stratum oriens and the alveus. The majority of the GAD-positive somata in stratum radiatum and stratum lacunosum corresponded to those of horizontal and other short-axon neurons. Granule and pyramidal cells did not appear to contain GAD-positive reaction product either in light or electron microscopic preparations. Some of the GAD-positive axon terminals formed symmetrical synaptic junctions with pyramidal and granule

cell somata and their dendritic shafts. The GAD-positive terminals forming axosomatic synapses corresponded in location to the endings of basket cells, known indigenous inhibitory interneurons. GAD-positive basket cell somata were seen to have many GAD-positive boutons on them, again emphasizing the potential importance of disinhibition in central information processing.

IMMUNOCYTOCHEMICAL APPROACHES TO RELATIONS AMONG NEUROTRANSMITTER SYSTEMS

GABA neurons obviously are in constant interaction with neurons liberating other transmitters or cybersnenes as well as with each other. One of the major objectives of current neurobiologic research is to delineate the relationships of various neuronal cell types to each other (18). The work in tracing the complexities of the synaptic relationships of GABA neurons has raised serious questions about the conceptual utility of formulating detailed hypotheses about information processing in the whole brain or in specific regions strictly on the basis of "classical" biochemical analytical approaches, by observing behavioral changes produced by lesions or pharmacological manipulations, or even by combinations of such approaches. Just as intracellular physiologic recordings from neurons must be carried out in morphologically characterized regions before they can reveal secrets of information processing, so now it is necessary to begin to perform chemical studies in more adequately described settings. It is necessary to develop detailed chemomorphologic maps of various brain regions in order to understand how neurons liberating the various putative transmitters and/or cybersnenes, known and still unknown, are related to each other. The work described on the immunocytochemical localization of GABA neurons has made it possible to begin to study the biology of an important class of identified local circuit neurons in various parts of the vertebrate central

nervous system. However, this is only one part of the story. It is necessary to be able to trace the connectivities of other types of inhibitory neurons (e.g., glycinergic) as well as those of the mainline neurons that, directly or through their collaterals, excite the local circuit neurons whose activities, in turn, serve to regulate the mainline neurons. Glutamate, aspartate, acetylcholine, and possibly some of the neural peptides may be major excitatory transmitters in vertebrate and invertebrate nervous systems, and glycine may be a vertebrate inhibitory transmitter. Eventually it will be necessary to identify the relationships of the neurons liberating these various substances by techniques similar to those developed for GABA neurons. The peptides, choline acetyltransferase, dopamine- β -hydroxylase, and tyrosine hydroxylase are already being visualized by immunocytochemical techniques in many laboratories. Currently what is lacking is knowledge of the rate-limiting enzymes involved in the biosynthesis of glutamate, aspartate, or glycine in the presynaptic endings of the neurons that may liberate them. Once these enzymes are identified with certainty, their purification, development of homologous antisera to them, and application of immunocytochemical procedures for their visualization in CNS tissue could be achieved rapidly.

Further refinement of present cytochemical and immunocytochemical techniques will be required. Procedures must be developed that are applicable to ultrathin sections so that it will be possible to visualize unequivocally two or more antigens, peptides, ions, etc. on one electron microscopic section; and eventually three-dimensional reconstructions will be possible.

GABA NEURONS AND FUNCTIONAL COORDINATION

How do GABA neurons, together with all other neural elements, participate in information processing in such a way that it is possible for particular organisms to respond adaptively to their environments in a manner compatible with survival and successful reproduction?

The ubiquity and extent of presynaptic endings of GABA neurons on various structures in the vertebrate nervous system, discussed above, are illustrated by the group of light micrographs of sections of rat CNS immunocytochemically stained for GAD in Fig. 3. The extensive studding by GAD-positive endings on neuronal postsynaptic surfaces in the nucleus interpositus (Figs. 3a and 3b), globus pallidus (Fig. 3f), cortex (Fig. 3g), and hippocampus (Figs. 3h and 3i) is particularly striking. After examining many such pictures and related electron micrographs, one gains the impression that he is looking at a highly restrained nervous system, the inhibitory neurons acting like reins that serve to keep the neuronal "horses" from running away. Elsewhere, evidence has been cited supporting the view that disinhibition may be one of the major principles of nervous system function (49). A major tenet of this hypothesis is that in behavioral sequences, innate or learned, pre-programmed circuits are released to function at varying rates and in various combinations largely by the disinhibition of pacemaker neurons whose activities are under the control of tonically active inhibitory command neurons, many of which may use GABA as a transmitter. According to this view, disinhibition is permissive and excitatory input to pacemaker neurons would have largely a modulatory role. Disinhibition, acting in conjunction with intrinsic pacemaker activity and often with modulatory excitatory input, appears to be one of the major organizing principles in nervous system function (32).

DISINHIBITION—A MAJOR ORGANIZING PRINCIPLE

Metaphorically, one may consider the pacemaker (or principal) neurons in various sectors of the CNS to resemble the classic pictures of Gulliver when he awoke to find himself restrained by ropes attached to him by the Lilliputians when he was asleep (Fig. 4). Cortical and hippocampal pyramidal neurons literally are studded with endings from GABAergic neurons, which presumably al-

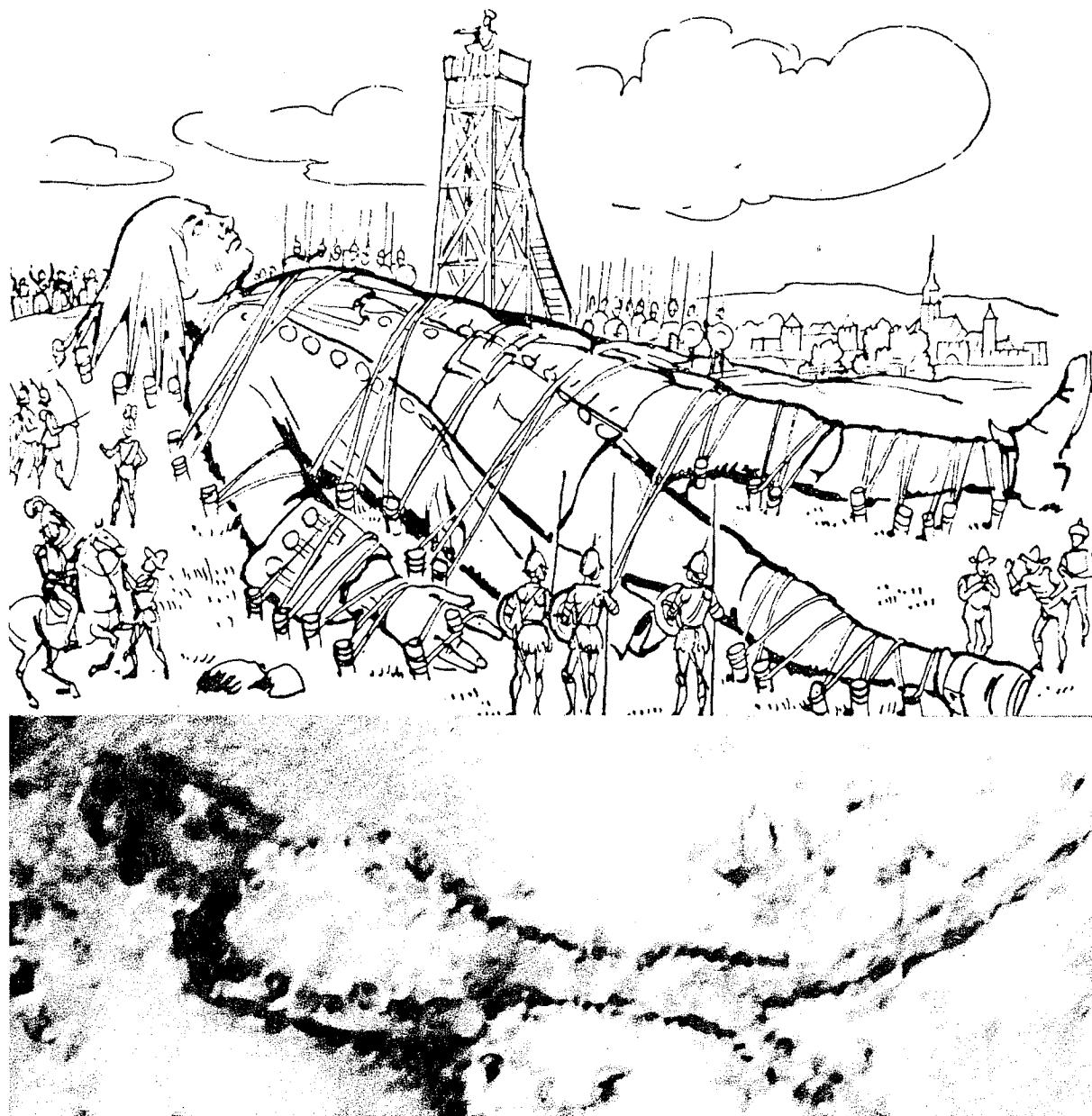


FIG. 4. The inhibited nervous system. An unretouched photograph taken with Nomarski optics of a neuron in the rat nucleus interpositus studded with GAD-positive terminals, presumably Purkinje cell axonal terminals, is placed below a picture of Gulliver, showing him when he awoke to find himself pinioned to the ground. The top picture is taken from the 1956 edition of volume 7 of *The Book of Knowledge*, The Grolier Society Inc., New York. (Figure prepared by Robert Barber.)

most entirely exert inhibitory effects. Not only are the endings of the aspinous stellate neurons distributed densely around the somata and dendrites of the pyramidal cells, but, at least in the case of the cortical neurons, they also have been located on initial axon segments where they may act as frequency filters. In addition, the GABA neurons themselves, which can be identified by somal, axonal, and

dendritic staining after prior colchicine injection, have GABA endings impinging on them (Fig. 5). One then gets a picture consistent with the idea that the pyramidal cells are tightly inhibited by GABA neurons, which themselves may be inhibited by the action of other GABA neurons in such a way that the inhibitory influence of some of them on the pyramidal neurons may be relieved, viz., both

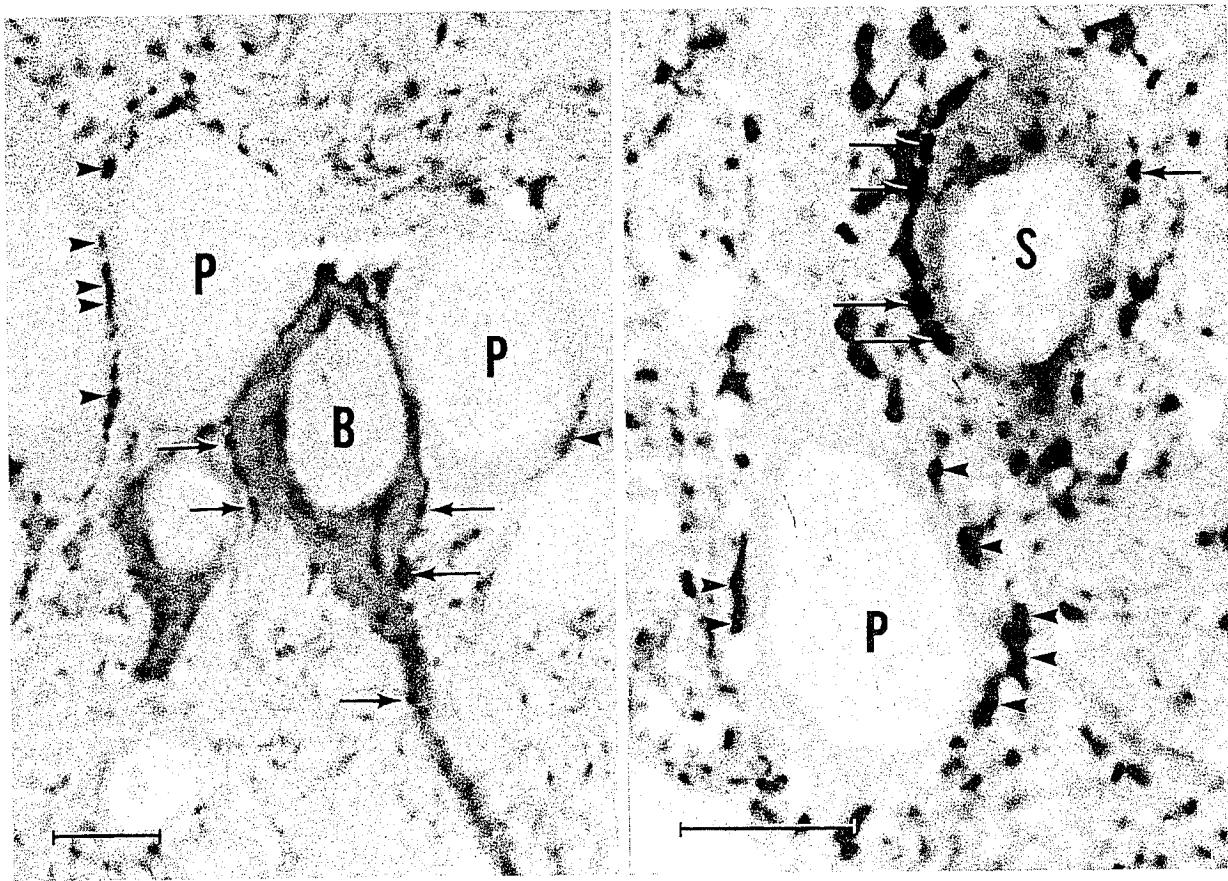


FIG. 5. **Left:** A presumptive basket cell (B) in rat hippocampus that stains positively for L-glutamic acid decarboxylase (GAD) and is, therefore, a GABAergic neuron. It is studded with numerous GAD-positive terminals (arrows). **Right:** Soma of a GAD-positive stellate cell (S) in layer V of the rat visual cortex that also is studded with GAD-positive terminals. In both micrographs, it is seen that somata of pyramidal neurons (P), which are not GAD-positive, are contacted by numerous GAD-positive terminals. Bar represents 10 μ m. (Figure prepared by R. P. Barber.)

inhibition and disinhibition play prominent roles in information processing in the cortex and hippocampus.

Similar data recently have been obtained in the spinal cord. Normally the principal cells, which possess the capacity for spontaneous firing and/or great sensitivity to excitatory input, may be held tightly in check by GABA neurons impinging on them. The information processing that goes on prior to the activation or release of the principal neurons may, to a considerable extent, be expressed via inhibition of the inhibitory nerves, that is, through disinhibition, releasing such neurons in one neural sector to fire at different rates and sequences to release suitable circuits in other levels of the nervous system. Communication

between neural stations and substations, we believe, takes place largely by the throwing of disinhibitory neural "switches," and the activities within them take place largely through preprogrammed, hard-wired circuitry. This may be the way in which information flows from sense organ to cerebral sensory area, thence through associative areas to the motor cortex and by way of the pyramidal paths to the final motor cells of the medulla and cord.

A GLANCE INSIDE NEURAL CIRCUITRY (OVERSIMPLIFIED AND PARTLY HYPOTHETICAL)

Since I generally favor a disinhibitory model for normal nervous system function and

disinhibitory mechanisms of epileptogenesis, it seems advisable to present some qualitative models of neural circuitry, not inconsistent with known neural arrangements, which might be useful in following our subsequent discussion.

Let us suppose that in a simple linear series of three neurons and a muscle fiber, neuron A controls through a single interneuron B the activity of a motoneuron C which, if left alone, could discharge spontaneously at a rapid rate, causing the muscle fiber to contract. Let us also suppose that interneuron B is a tonically active inhibitory neuron from which, in the absence of input from the first neuron in the series, A, an inhibitory transmitter is liberated at such a rate that the membrane potential of the motoneuron is held at a level below the firing level, and the muscle fiber does not contract. Neuron A is presumed to be a phasically active inhibitory neuron. When it is caused to discharge on interneuron B, inhibition from the latter on motoneuron C will be decreased. As a result, the motoneuron will fire; this, in turn, will cause contraction of the muscle fiber to take place.

Let us now substitute for the motoneuron and muscle fiber in the above model an entire simple neural circuit (Fig. 6). The principal or pacemaker neuron (P) for the operation of a particular circuit then would be analogous to the potentially spontaneously active motoneuron in the preceding model, and would be under the restraint of the tonically active inhibitory interneuron, I_t . A phasically active inhibitory neuron, I_p , when activated, would inhibit I_t , making it possible for P to fire by relieving it of the tonic inhibition exerted by I_t , i.e., by disinhibiting it.¹ Excitatory afferent input impinging onto I_p neurons from neighboring neuronal circuits or from circuits in other neural sectors, in some instances coming via dendritic spikes propagated electrotonically to the soma, would help release the P neuron for action. In reality, there would be

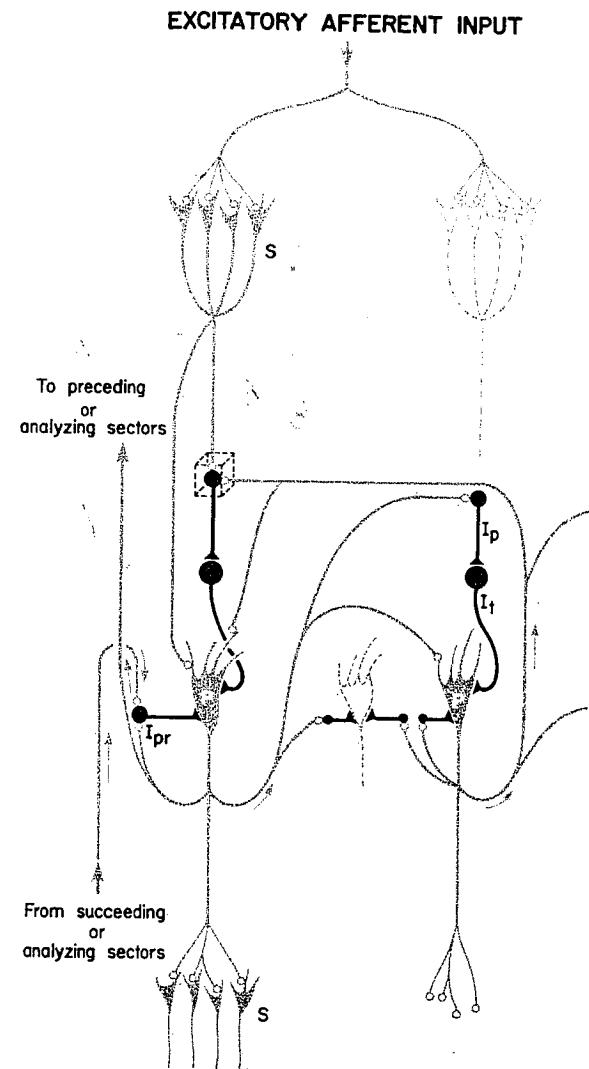


FIG. 6. An outline of "minimal" basic neural circuitry. The details of this model are discussed in the text. P, principal or pacemaker neurons; I_t , tonically active inhibitory neurons; I_p , phasically active inhibitory neurons; I_{pr} , phasically active inhibitory neurons involved in disynaptic recurrent inhibition; S, excitatory stellate neurons.

multiple excitatory and inhibitory inputs onto both the P and I_t neurons; and a particular I_t neuron might inhibit a number of P neurons, as probably occurs in the case of hippocampal basket cells or retinal amacrine cells. However, the model requires that, in most instances, decrease or cessation of the inhibitory signal from the I_t neuron would be a necessary, but not always sufficient, condition for the firing of the P neuron. The latter might begin to fire spontaneously when partially or

¹ Figure 5 shows examples of GABAergic neurons with GABAergic endings on them in the rat cerebral cortex and hippocampus.

completely relieved of inhibition exerted by the I_t neuron; or excitatory input to the P neuron might be required for it to depolarize to the firing level, even when the tonic inhibitory influence on it has been decreased or even completely removed. In the latter instance, less excitatory input would be required to fire the P neuron in the absence of inhibition by the I_t neuron than in its presence.

After a stimulus pattern first is experienced by an organism, many neurons in various through-put sectors² of the CNS are affected, either activated or inhibited. I would like to propose that the consequence of effective afferent neural input to any sector from any other sector of the nervous system, starting with receptors, is the change in activity by combinations of disinhibition and excitation of groups of excitatory P neurons (Fig. 6).³ The individual P neurons in these groups control, through their axonal endings, genetically determined programs of neural activity in satellite neurons (S)⁴, excitatory interneurons,

which in turn signal P neurons in other neural sectors and/or effectors (muscles, glands). The frequency of firing of the P neurons would at any given moment determine the exact details of such programs, since the presynaptic impulses of the main axons invade axonal branches, the activities of which vary with the frequency of firing of the main axon. Frequency-dependent differential channeling of information can take place at points of axonal branching (22). Although all the branches of a particular neuron usually are believed to liberate only one true neurotransmitter,⁵ the effects of this liberation might be either excitatory or inhibitory, depending on the nature of the postsynaptic receptors affected by the particular transmitter. Thus the P neurons integrate the incoming signals at all levels from dendritic endings to initial axon segment and through their activities express frequency-dependent aspects of genetically coded neural programs that are carried out by the satellite neurons. The satellite neurons of active circuits would largely serve as the means of effective communication with other neural sectors.

There also are inhibitory interneurons (I_{pr}) that are phasically active and furnish disynaptic recurrent inhibition. These are activated by recurrent collaterals of the P neurons and can exert hyperpolarizing postsynaptic inhibition on the same P neurons by which they are activated as well as possibly on other P and S neurons nearby. They also may exert depolarizing presynaptic inhibition, where it exists. Some of the above relations are not illustrated in Fig. 6.

In recent years, as a result of a confluence of morphologic and physiologic observations, the concept has arisen that there are aggregates of mutually interrelated neurons, ranging from a few up to perhaps several thousand in number, in the cortex and elsewhere in the CNS, that may serve as modules or "power packs." I would like to speculate that in each

² My concept of such a sector does not correspond exactly to a particular classically designated anatomic structure, but rather includes excitatory projection neurons and the excitatory interneurons on which they impinge. For example, specialized thalamocortical cells and their excitatory cortical satellites (spinous stellates) are considered a "sector." In contrast to the through-put sectors, the projection neurons of analyzing sectors, such as Purkinje cells of the cerebellar cortex, frequently are inhibitory. The latter will not be discussed in detail here.

³ Throughout this discussion I have in mind the cortical synaptic relationships described in Chapter 3. Thus candidates for excitatory interneuronal satellite neurons (S) might be the cortical spinous stellates. Some aspinous stellates may be the phasically active inhibitory (I_p) interneurons and some aspinous stellates and basket cells may be the tonically active inhibitory command (I_t) neurons (see Fig. 6). The pyramidal neurons are presumed to be the projection neurons (P). Some pyramidal neurons from other sectors, possibly important largely for extremely rapid emergency communication, may synapse directly on P and I_p neurons without mediation of satellite cells. Some such inputs may go directly to satellite neurons (not shown in Fig. 6). The latter is most evident in the case of the spinal motoneurons, which in primates receive direct input from pyramidal tract neurons. I recognize, of course, that details of circuitry differ from one neural region to another, as discussed so elegantly and understandably by Shepherd (61).

⁴ Examples of S neurons might be the spinous stellate cells of the cerebral cortex or the spinal motoneurons.

⁵ Recent data suggest that some neurons may liberate a true transmitter and also a cybernene, for example, GABA and substance P.

neural sector, from receptor to cortex and from cortex to effector, there are redundant, functionally similar (but not identical) groups of neural modules. Although the neurons within a given module, or even in several modules, may have had a clonal origin, unique aspects of location and experiential history would have given rise to a variety of structural and functional individual differences among them at any subsequent period of observation. At each functional level we presume there to be classes of modules that respond to given inputs which are first determined at the level of receptor transduction and first-order neurons. In turn, these modules signal the frequency and intensity of input received by them in a manner interpretable by related modules in other sectors. Collaterals from their active P cells are presumed to impinge on inhibitory interneurons lying between the neural elements of different modules in such a manner that after the onset of activity, each module would have a tendency to create an inhibitory surround in which adjacent, or possibly even interpenetrating, modules would tend to be held in a state of inactivity or reduced activity. An active cortical "column" could consist of vertically communicating modules and might be surrounded by inhibited "columns." Units for different, or even antagonistic, functions frequently might be located side by side, exerting an influence on one another. Thus the activities of modules subserving a particular function could inhibit not only some functionally redundant modules but also those involved in incompatible responses, viz., flexion-extension, eating-satiety, waking-sleeping, warming-cooling, attacking-fleeing.

A module has among its various cellular components groups of spatially distributed, functionally redundant P neurons and their associated S neurons, as shown in Fig. 6. Let us assume that, as a result of existing patterns of synaptic connectivity, a particular class of impulses would begin to arrive in this region largely from the satellites of P neurons of other sectors and that the two active P neurons shown would be potentially capable of re-

sponding to the arriving input. Excitation would take place via synapses directly on the P neurons, presumably largely on dendritic processes. Disinhibition is shown to occur by excitation of the phasically active inhibitory interneurons, I_p , that act directly on the soma or proximal dendrites of the I_t neurons, the tonically active inhibitory neurons that form synapses largely on the soma and initial axon segments of the P neurons. The P neurons are depicted as communicating with each other via axon collaterals that produce both excitation and disinhibition. Which of such P neurons in a given sector would have their activities changed, increased or decreased, during the first submillisecond instant of arrival of a new input would be determined by their eligibility at the time, depending on factors such as the temporal sequence of their activation, their exact response characteristics, the degree of membrane polarization, and metabolic state. When inactivation of the I_t neuron has taken place, the P neurons are released to fire and send impulses down their axonal branches and into their collaterals. Impulses arising in the P neurons result in the activation of their satellite neurons and, through their collaterals, in the activation of phasically active inhibitory interneurons, I_{pr} , which impinge back onto the P neurons and provide for inhibitory phasing of their activity. The active P neurons are shown to inhibit their neighbor, possibly a member of another module, via inhibitory interneurons. The P neurons belonging to the same module also could be excited and disinhibited, respectively, by inputs from collateral branches from each other, as indicated. Collateral branches of the P neurons feed back to preceding neural sectors in the sequence, signaling to decrease or stop the input from them. Such collaterals are presumed to exert their effects mainly by activating I_{pr} neurons, in a manner similar to that shown in Fig. 6, for excitatory inputs coming back from the sector to which the P neurons communicate through their satellites. The P neurons eventually cease activity when the combined effects of the excitatory inputs to them

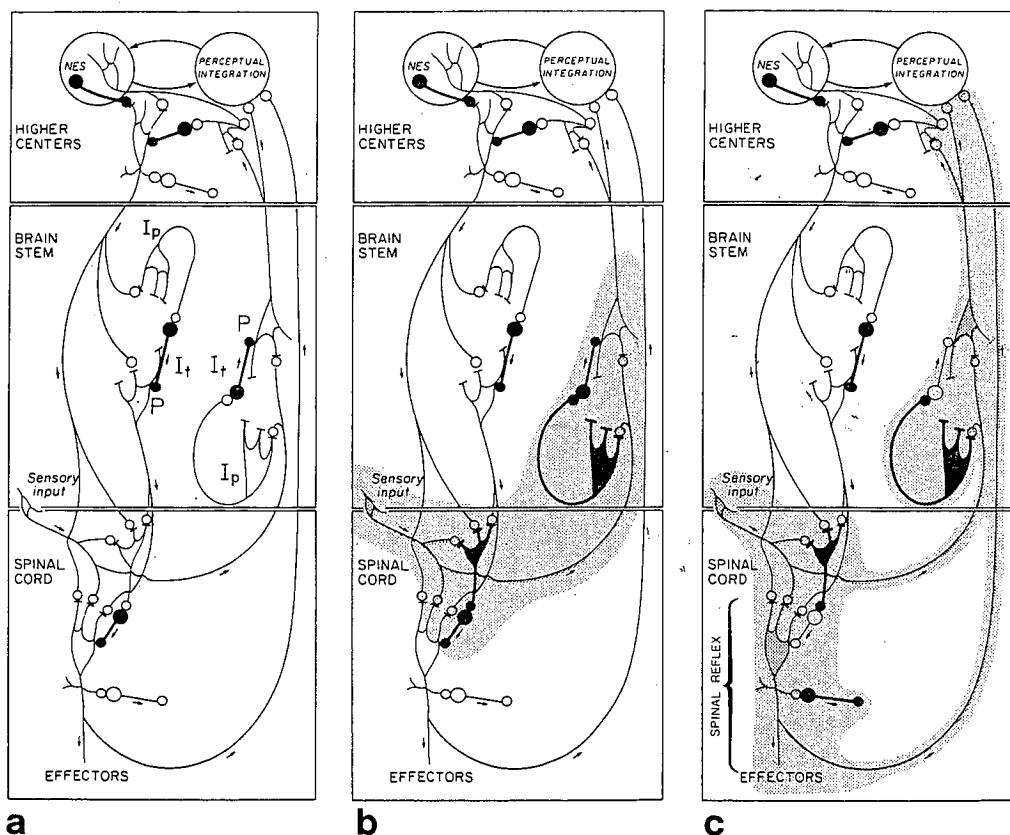


FIG. 7. Communication between neural sectors. A neuronal model for relationships between spinal cord, brainstem, and higher centers in the vertebrate nervous system emphasizing the controls exerted by tonically active inhibitory neurons (I_t). Details are discussed in the text. Active inhibitory neurons are shown in solid black.

have decreased below a certain critical level and when their I_t neurons again become fully active.

I now postulate that, with use, connectivity increases could take place selectively at excitatory synapses on the I_p neurons that inhibit the I_t neurons. The origins of these inputs are indicated to be largely satellite neurons of P neurons from other sectors (e.g., the spinous stellate cells of the cerebral cortex that receive thalamic and/or callosal afferents) and excitatory collaterals from P neurons in the same sector. Such a formulation avoids the occurrence of plastic changes taking place at synapses arising from or impinging directly on the P or I_t neurons. This would have the advantage of not tinkering with genetically determined "hard-wiring" of the nervous system and would prevent interference with basic communication codes that might exist between the I_t and P units. This formulation places the burden of modification by experi-

ence on synapses that are one or more synapses removed from the projection neurons in the mainline channels of communication. In Fig. 6, the P neurons in a given input are postulated to have mutually disinhibitory connectivities. If such connectivities should increase with use within modules and even possibly between some of them,⁶ a repetition of a given input pattern at a subsequent time would have a tendency both to release a greater number of P units for synchronous action and to increase their activity to a greater extent than at the beginning of the first exposure to it.

COMMUNICATION BETWEEN NEURAL SECTORS

In order to simplify the picture, only single excitatory principal or pacemaker (P), toni-

⁶ See Roberts and Matthysse (53) for discussion of formulations relevant to synaptic connectivity.

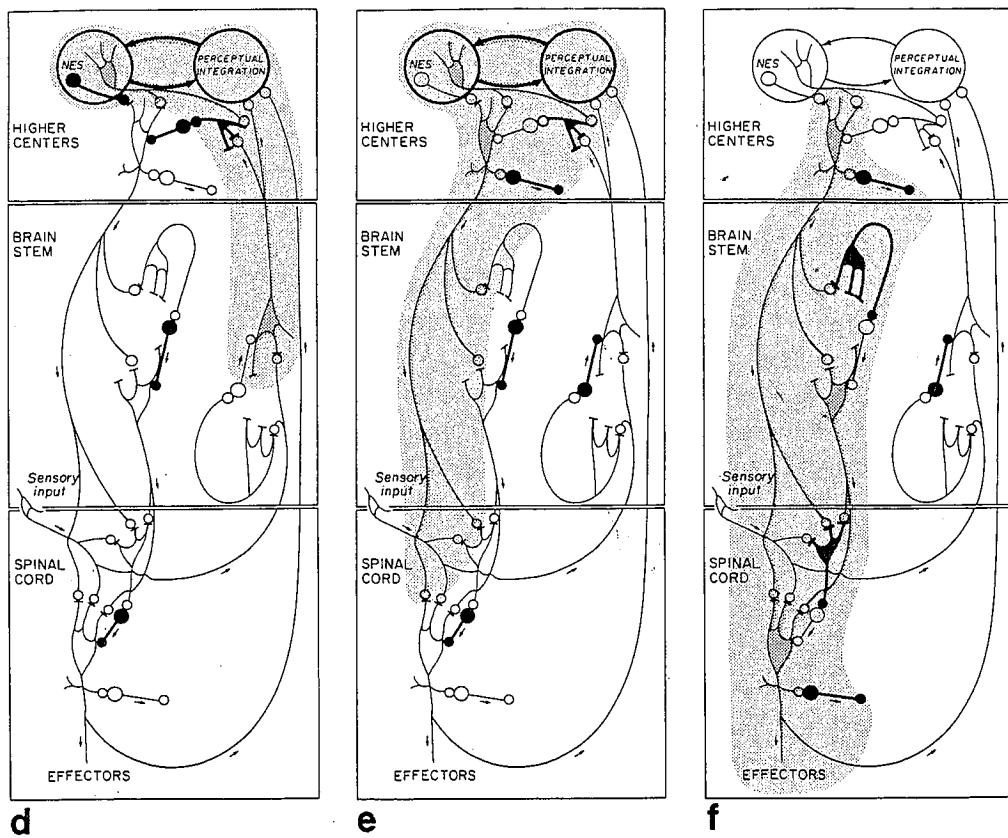


FIG. 7. Continued.

cally inhibitory command (I_t), and phasically inhibitory (I_p) neurons are shown at each neural level. Figure 7a and 7b-f show schematized prestimulus and poststimulus versions, respectively, of "minimal" basic neural circuits in higher brain centers, brainstem, and spinal cord. In all instances on the diagrams, active neurons are represented in solid form and inactive ones are shown only in outline. Most of the I_t and I_p neurons are presumed to employ GABA as transmitter, although glycine probably also is employed, particularly in the cuneate nucleus and ventral regions of the cord. That at least some of the P cells and their excitatory satellites (not shown) may use glutamic or aspartic acids or acetylcholine as transmitters is compatible with many indirect data but is not proven. In the case of motoneurons, the transmitter is known to be acetylcholine.

Excitatory information entering from the receptors via primary afferent fibers can go to neurons located either in the spinal cord

or in the brainstem, increasing their probability of firing (Fig. 7a). Sensory input comes in via excitatory endings that may impinge directly on dendrites of P neurons and also on I_t neurons lying in the immediate neighborhood in such a way as to disinhibit the P neurons by liberating them from the tonic inhibition of the I_t neurons (Fig. 7b). The identity of the chief excitatory transmitter(s) for the primary afferent neurons has not yet been established with certainty, although glutamate and substance P are two of the candidates. Some of the indigenous GABA interneurons that are depolarized by the afferent input could depolarize adjacent sensory endings through axoaxonal connections (presynaptic inhibition and dorsal root reflex) and could hyperpolarize postsynaptic sites on other inhibitory interneurons (presynaptic facilitation) or on P neurons (inhibition). Primary afferent inputs may affect directly the cholinergic spinal motoneurons in such a way that their discharge would elicit a spinal reflex prior to

detailed processing of the incoming signals in CNS regions beyond the spinal cord segment involved (Fig. 7c). The latter situation must be clearly distinguished from that in which motor units would be used as part of a coordinated behavioral sequence resulting from a processing of the incoming stimulus pattern in the higher centers of the CNS.

It is obvious that the simple spinal reflex is not an adequate model for CNS function. Indigenous inhibitory GABAergic neurons are most numerous in the substantia gelatinosa and in the upper laminae of the dorsal horn of the spinal cord, although they are distributed throughout the cord, and endings of GABA neurons are found even on somata of motoneurons. Inhibitory interneurons possibly employing glycine as transmitter seem to be richer in the ventral portions of the spinal cord. Indirect evidence suggests that glutamate and aspartate may be excitatory transmitters in the cord and that the latter may be the major transmitter of the polysynaptic pathways (52). In mice and rats, a system of fine longitudinally oriented noradrenergic fibers is found in the substantia gelatinosa; in the cat, morphologically similar terminals probably contain serotonin. Somatostatin and substance P also are present in nerves in this region.

Although it will be some time before the details of the synaptic and/or cybernene relationships between all of these neuronal types will be elucidated, it has been shown that indigenous spinal circuits in the cat can be released for action, at least in part, by the disinhibitory action of descending norepinephrine (NE) fibers originating from cells lying in the "mesencephalic locomotor region." In the spinal cord, iontophoretically administered NE has been shown to have inhibitory effects. NE-mediated inhibition in spinal trigeminal neurons has been found to originate in the locus ceruleus. Acute spinal cats ordinarily show neither postural nor locomotor activity. However, after intravenous administration of clonidine, a drug that passes the blood-brain barrier

and specifically stimulates central α -noradrenergic receptors, it was possible to elicit walking behavior on a moving treadmill at a speed that could be adjusted by the speed of the treadmill (17). In the best preparations "this locomotion looks normal to the eye with smooth alternating movements in all joints." Thus stimulation of α -noradrenergic receptors on neurons in the cord, combined with stimulation by a treadmill, can release the expression of neuronal programs for coordinated postural control and locomotion that are located entirely in the cord. It is possible that NE somehow acts to relax the tonic inhibitory effects of the I_t cells in the cord, possibly by inhibiting them and thus helping release P cells for function. Cerebellar Purkinje cells also may be inhibited, possibly nonspecifically, through the widely ramifying NE-releasing endings of nerve fibers which have their cells of origin in the locus ceruleus. Thus the effects of inhibitory neurons utilizing GABA as a transmitter may be decreased by other GABA neurons and/or by noradrenergic neurons. In the complex arrangements of different regions of the CNS, various combinations of inhibitory neurons can act on each other.

Figure 7b and 7c shows that the sensory input can enter the brainstem, where it acts directly on the P neurons and on the I_p interneurons lying entirely within the brainstem. The latter can inhibit the tonically active I_t neurons that are holding intersystem P neurons in check. The excitability of brainstem neurons is believed to be maintained by inflow of impulses along collaterals from specific afferent pathways. This action could result in the activation, largely through disinhibition, of the P neurons in the brainstem that relay to the higher brain centers (Fig. 7d). At least some of the brainstem P neurons probably use acetylcholine as transmitter. Ascending pathways from the reticular formation of the brainstem are important in increasing the background activity of neurons in higher brain centers. When the connections between the brainstem region and the cortex are cut, either

the activity of cortical neurons stops completely or the frequency of discharge becomes much lower than in the brain of an intact animal. Stimulation of this region in an intact animal increases the activity of cortical neurons. Impulses from the brainstem may merely serve to help release cortical units for firing in their own characteristic fashion. Pathways from the brainstem probably are much more important for maintaining cortical activity than those from the specific sensory nuclei. If the primary inhibition within the cortex is exerted by a system of tonically active GABAergic I_t interneurons, then the excitatory input from the satellites of the P neurons from the brainstem, the excitatory spinous stellates, could act in such a way as to depolarize the disinhibitory I_p neurons and the dendrites of cortical P cells, and this, in turn, would result in the activation of cortical P cells (Fig. 7d and 7e). According to the above scheme, brainstem P neurons, activated by incoming sensory input, send signals from the brainstem region to the higher centers, releasing the activity of cortical pyramidal neurons.

Figure 7d and 7e shows that the input from the brainstem occurs both to cortical P neurons and to centers involved in perceptual integration, such as the hippocampus, cerebellum, basal ganglia, and association cortex. It also is indicated that, in general, the input from brainstem neurons is not sufficient in itself to release a preprogrammed effector circuit. Instead, it is suggested that perceptual integration must take place within the analyzing regions of the nervous system and that the neuroendocrine servo system (NES) must send either a "go" or a "no go" signal. I believe that the NES receives inputs representing the emotionally significant aspects of the total stimulus pattern, external and internal, summates them, and then may generate neural signals which cause the organism to act by releasing or disinhibiting behavioral options already available to it. The activity of the NES also causes the release of chemical signals in the brain, and systemically from neural and

endocrine structures, in amounts which facilitate the implementation of the neural ones. If a decision is reached that a particular behavioral option is not to be employed, then the disinhibitory signals will not go out to the cortical P neurons related to the control of that behavioral option. As a result, these neurons will not discharge at all or, at least, will not increase their basal rate of activity. This possibility is indicated in Fig. 7d, but not followed through in detail in the diagram. On the other hand, if a decision is reached that a particular behavioral option should be employed, then excitatory signals coming from the NES could act via I_p neurons on the tonically active I_t neurons and also directly onto the P neurons, releasing them for action.

Figure 7e and 7f shows the results of the disinhibition of a P neuron in a higher brain center. It is presumed that such a neuron sends excitatory signals to the brainstem, disinhibiting a descending P neuron. The latter communicates disinhibitory and excitatory influences through the pyramidal tract, releasing neurons in the spinal cord from inhibition by tonically active I_t neurons therein. Finally, activities of combinations of motor neurons cause those effectors to respond which are involved in the particular behavioral option that has been selected. It also is indicated on the diagrams that the P neurons in the cortex and the motoneurons in the spinal cord can excite inhibitory interneurons, which would tend to create a lateral inhibitory surround during their activity.

The shading in Fig. 7b-f indicates that cybernetes are released from their neurons regionally in a manner coordinated with the functional needs of the neurons in the activated circuits. The cybernete-releasing neurons can be thought of as belonging to a system parallel to that of the information-transmitting circuitry. It is as if a signal could be given to a piece of sequentially active machinery to begin to work and simultaneously to an oiling system that would supply oil in small amounts, just before use and during use, to

those parts of the machine that will be used and in amounts related to the anticipated extent of their use.

Although the preceding formulation has been made with the premise that nervous systems operate in a linear fashion, the strategy that appears actually to have been employed by nervous systems is for P neurons in one neural sector to inform, directly or through their satellites, neurons in one or more other neural sectors about their activity. Minimally, a bifurcation of the flow of neural information from a particular sector takes place into direct through-put channels and also into those leading to analyzing regions such as the cerebellum, hippocampus, basal ganglia, and association cortex. In the analyzers, information arriving from several sources may be integrated and the output reflecting this analysis, with variable time delays, then plays on neural elements in the direct channels to adjust the activity therein so that it is more compatible with activity elsewhere in the CNS in helping achieve adaptive responses to the environmen-

tal exigencies being faced at the time. The cerebellar cortex is one such region of analysis, and its output via inhibitory Purkinje cells may serve to adjust the phasing of various motor activities through its rich and powerful inhibitory output onto recipient cells in several other regions. It is suggested that inhibition coming from cerebellar Purkinje cells, from cells projecting from the globus pallidus to the ventrolateral thalamus, and possibly from other analyzing sectors as well, creates a negative imprint on the flow of excitatory information in a manner reminiscent of punching holes in a player piano roll or a computer card (Fig. 8). The above also suggests that there may be two basically different kinds of neural subsystems in the vertebrate CNS. There are those whose projection neurons are excitatory (the through-put systems) and those whose projection neurons largely are inhibitory (the analyzing systems). The latter may be largely limited to regions such as the cerebellar cortex, the striatum, globus pallidus, and association cortex.

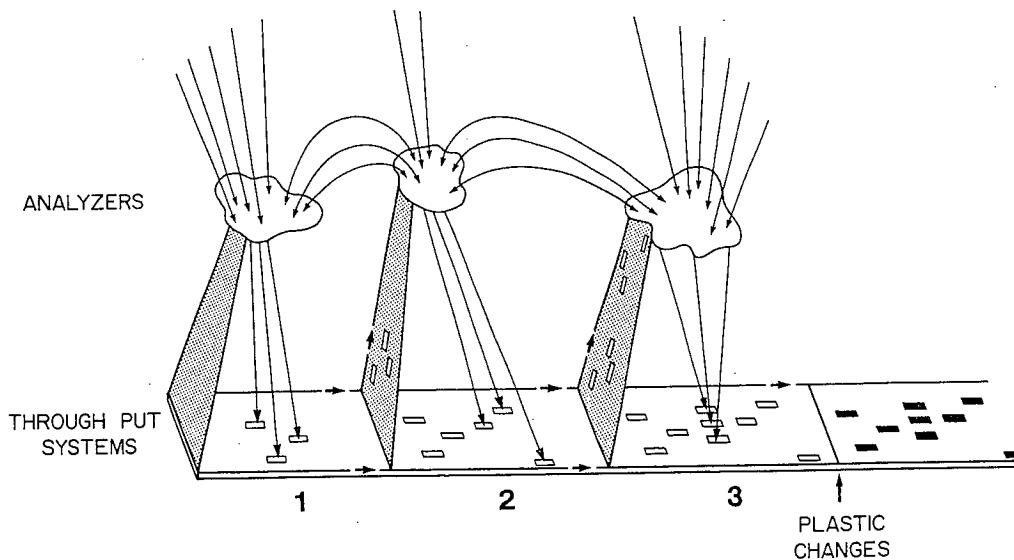


FIG. 8. A model for relationships between through-put systems and analyzing sectors. Minimally, a bifurcation of the flow of neural information from a particular sector takes place into direct through-put channels and also into those leading to analyzing regions such as the cerebellum, hippocampus, basal ganglia, and association cortex. The arrow indicates that plastic changes (increases of connectivities) occur at some synapses when it is signaled that an appropriately adaptive response to a new situation has been achieved by the organism. See Roberts and Matthysse (53) for pertinent discussion of plastic changes.

INADEQUACY OF INHIBITION AND DISEASE: HOW SEIZURES MAY OCCUR AND SPREAD

The successful or adaptive operation of a nervous system requires a coordination of neuronal activity in order to prevent too-frequent or too-infrequent firing of preprogrammed circuits of behavioral options spontaneously or maladaptively. Under a variety of environmental circumstances, internal and external, an organism must maintain within physiological limits the rates of operation of continuously needed neuronal circuits, such as those required for cardiac function, respiration, and maintenance of blood pressure. When gross malfunctions of the coordination of inhibitory and disinhibitory neuronal systems or between the neuronal and cybernene systems occur, there may result lethal effects through either generalized seizures or cessation of operation of some vital function; alternatively, some obviously severe neurologic-physiologic dysfunctions may occur.

When there is persistent incoordination between the GABA system and other neurotransmitter and cybernene systems, for whatever reason, the defect might be restricted to a local brain region, might include several regions, or might be global throughout the CNS. A critically placed local incoordination may have drastic reverberations in the whole nervous system, as found in grand mal seizures arising from focal cortical lesions. Under relatively simple environmental conditions, the nervous system in such individuals could function in an apparently adequately adaptive manner, which might appear to be in the normal range. As the complexity and intensity of environmental inputs is increased, there would be a correlated increased degree of incoordination. Then those subsystems in the nervous system that are most poorly controlled will tend to break down under the stress and produce symptoms that are consequent to such a breakdown.

Let us suppose that for some reason in the

entire brain or in specific regions, tonically active inhibitory GABA neurons have a considerably lower than normal effectiveness on their recipient neurons, which themselves are normally effective; or, alternatively, that the GABA neurons are normal and the neurons they ordinarily control are less than normally responsive to GABA or are potentially hyperactive because of intrinsically arising or extrinsically produced membrane changes. With increasing excitatory input, there would be an increased tendency to release pacemaker neurons. More than normal numbers of behavioral options or inappropriate ones (e.g., behavior disorders, mania, schizophrenia) would be released; choreic movements, seizures, or spasticity might occur; there might be hypersensitivity to visual, auditory, tactile, olfactory, gustatory, or pain stimuli. For example, with regard to the latter, a recent report showed that a variety of epileptic patients showed a marked hyperosmia for three classes of odors whether or not the patients were receiving antiepileptic drugs (8). A number of recent experimental results suggest that GABA neurons may play important roles in control mechanisms in various hypothalamic centers. Thus, if specific hypothalamic regions were affected, greater than normal degrees of changes in responses might be observed, for example, in emotional reactivity, cardiac and respiratory functions, blood pressure, food and water intake, sweating, galvanic skin response, insulin secretion, liberation of gastric acid, and motility of the colon.

Virtually from the beginning of physiologic and pharmacologic observations of the GABA system it has been conjectured that decreases in the efficacy of the GABA system could result in convulsive seizures. It certainly seems possible that in schizophrenia, and in Huntington's and Parkinson's diseases, as well as in other disorders, deficiencies might exist in the relative numbers of GABA neurons in specific brain areas, or that there might be defects in the structural or functional relationships of GABA neurons with other neurons, which

either might inhibit or excite them, or which they themselves presumably might inhibit.

It has been emphasized in the preceding discussion (see Figs. 6 and 7) that I believe that the permissive element in the activity of the P neurons is the release from inhibition by the tonically active I_t neurons and that the excitatory input directly onto the P neurons generally might not be sufficient to release them for activity during normal nervous system function. Let us now suppose that for some reason the I_t neurons in a particular neural sector have a considerably lower than normal effectiveness on their recipient P neurons. As the intensity of inputs is increased from some low basal level, there would be a corresponding increase of excitatory influences on the I_p neurons and on the P neurons themselves, resulting in release of a greater number of P neurons than ordinarily expected for the given input. Depending on the extent of impairment of I_t -P cell interaction, the intensity of input, and the compensatory adjustments possible through action of the cybernene systems, a point could be reached at which one of the P cells in the sector that is no longer capable of being restrained by its I_t neuron would begin to fire spontaneously, occasionally or in a rhythmic fashion characteristic for that neuron. The input from the excitatory collaterals of this neuron could add to the excitatory and disinhibitory influences on P neurons in the vicinity, on which only a tenuous hold may be maintained through their defective relationship with I_t neurons, recruiting them also into synchronized discharge pattern.⁷ This could continue until vir-

tually all of the available P neurons in the modules of a given sector are involved in responding to their pertinent inputs. Increases in connectivity could occur at some synapses, thus facilitating recruitment in future rounds of activity. Eventually the effects of the spread of such activity may be sufficiently great to cause electrographically and behaviorally detectable seizures, as discussed in the following section.

The above considerations may be relevant to the recently studied phenomenon of kindling, in which there has been shown to be an eventual development of motor seizures in limbic and other brain sites following repeated low-level electrical stimulation which originally had no effect on behavior and did not cause electrographic discharge (19). The kindling phenomenon usually is a result of abnormal repetition of unusual stimulation. According to our model, either facilitation of excitatory synapses on the disinhibitory I_p neurons or weakening or destruction of the I_t neurons would lead to increased activity and seizure susceptibility. Kindling phenomena would be possibly expected to be associated with use-dependent plastic changes and trauma as a result of injury or the application of alumina cream or cobalt, for example, with neuronal destruction. Also, genetically determined inadequacies in neural inhibition at one or more sectors from receptor to cortex could result in the evocation of seizures by photic, auditory, olfactory, thermal, and other sensory cues in animals and man.

A multiplicity of chemical and physical changes probably occur in neural membranes and neural regions that undergo the abnormally intense activity found during seizures, and metabolic exhaustion and even physical destruction of some of the I_t neurons in the

⁷ Although recruitment probably occurs through the more commonly considered pathways involving axon collaterals, in considering the firing of pyramidal neurons and their synchrony in seizure discharges, one also must give heed to the possibility of electrotonic interactions among them through gap junctions. Although they have not yet been thoroughly analyzed at the physiologic and ultrastructural levels in most regions of the CNS, there is reason for thinking that gap junctions may be involved importantly in complex information processing and that there may be conditions under which a relatively small number of unrestrained pyramidal cells may through such contacts also help enlist the cooperative firing of a large

number of other pyramidal neurons. A possibility also exists that the formation and dissolution of electrotonic junctions between neuronal processes may be occurring continuously and that alterations of environmental factors, e.g., extracellular levels of Ca^{2+} ions, may play a role in determining the extent and stability of such junctions at any time (2).

vicinity might take place with continued seizures.

NERVE ACTIVITY, METABOLISM, AND SEIZURES

A key question not dealt with above relates to the origin of the apparent discontinuity that exists between the increase in normal activities of neurons and their explosive hypersynchronous involvement in seizure discharges, whatever the nature of the problem that causes neural incoordination to go beyond a compensatory range. It does not appear that this question can be answered from neurophysiological observations alone. Are there any known chemical changes accompanying intense neural activity that may be closely related to the abrupt transition that takes place? My inclination is to point my finger to ammonia as a possible chemical culprit. Ordinarily, after neurons are excited, energy utilization is increased and the intracellular levels of creatine phosphate and ATP fall momentarily during the time of ionic reconstitution, after which these levels are restored through coupled oxidative processes. In the microenvironment of the activated membrane during this period, there may be an increase in ammonia content, most of which may arise from the deamidation of adenylic acid (59). The inosinic acid formed from the latter is subsequently reamidated via a series of reactions (the purine nucleotide cycle) in which the nitrogen ultimately comes from amino acids that are capable of being transaminated with α -ketoglutarate to form glutamate. The ammonia formed during nerve activity can be removed by conversion to glutamate (action of glutamate dehydrogenase) and glutamine (action of glutamine synthetase) or by diffusion into extracellular space and subsequently into the bloodstream. In the physiologic pH range, ammonia exists mainly in the form of the ammonium ion (NH_4^+), but a part, perhaps less than 1%, exists in the undissociated form, NH_3 ($\text{pk} = 9.5$ in H_2O at 38°C). The undissociated form diffuses through membranes much more rapidly than

O_2 or CO_2 . Such a fast diffusion of NH_3 across the brain cell membrane usually prevents the accumulation of brain cell NH_4^+ . Ammonia entering the blood supply to the brain from extraneuronal sources is probably largely detoxified by conversion to glutamine in astroglial cells. But it seems likely that the ammonia arising as a result of nerve activity normally is removed at local sites of formation near neuronal membranes.

If neural activity becomes intense and prolonged, greater and more prolonged decreases in creatine phosphate, ATP, and α -ketoglutarate levels would take place and more ammonia would accumulate locally, increased amounts persisting for longer periods because of the depletion of ATP and α -ketoglutarate (59). The ammonia would diffuse to the region of membranes and through them into the immediate extracellular environment exerting a number of actions, all of which might lead to the final crescendo of seizure activity. Most importantly, ammonia can inactivate the chloride pump in neural membranes, thus negating to a large extent the ability of GABA and any other neurotransmitters that open chloride channels to hyperpolarize postsynaptic membranes (28,30,37,42). In the case of GABA, this might reduce perhaps to one-tenth the inhibitory potency of a given amount of synaptically liberated GABA (28). The remaining portion of the inhibitory effect would reside with the shunting current occurring as a result of the opening of the Cl^- channels. Thus ammonia could largely negate the postsynaptic inhibitory efficacy of GABA neurons without having any effect on their ability to produce or release GABA or in any way affecting the interaction of GABA with its postsynaptic receptor and the consequent increase in Cl^- conductance. In addition, NH_4^+ , which like K^+ is a powerful depolarizing agent, would act in conjunction with other excitatory influences, among which might be increased extracellular K^+ , to increase the tendencies of conductile components of neurons to undergo repetitive firing and to greatly augment polysynaptic EPSPs and axon terminal burst-

ing. The action of methionine sulfoximine, a potent convulsant, probably is largely attributable to its ability to inhibit glutamine synthetase and thus greatly decrease the rate of removal of ammonia close to its neural sites of formation.

My proposition, then, is that ammonia, formed in small amounts and removed rapidly during normal nerve activity, may rise greatly during a prolonged period of intense neural activity. Eventually, sufficiently high local concentrations may be reached in the vicinity of inhibitory synaptic junctions so that the activity of the Cl^- pump would be blocked. The resulting inactivation of the system producing hyperpolarization would act like a switch, removing almost all inhibition and converting a cybernetic system into one with positive feedback only. A diagrammatic representation of a portion of a module without inhibition is shown in Fig. 9.

Inhibitory GABA neurons would also be caused to fire at grossly excessive rates during the intense activity of a seizure, possibly liberating such large amounts of GABA locally that the ability of transport and metabolic systems to remove it would be exceeded. After the cessation of a seizure and the decrease of ammonia level below that inhibiting the Cl^- pump, the excess GABA in the vicinity of membrane receptors could create a prolonged hyperpolarization until its removal would take place. Such a local accumulation of GABA also might decrease conductile properties of spike-generating cells since, at least in the case of unmyelinated axons in mammalian peripheral nerve trunks, GABA has been shown to decrease spike amplitude and depolarize the nerves (7). Indeed, prolonged postictal inhibition is commonly observed after seizures.

Insofar as it is possible to assess the current physiologic literature, it appears that in a number of experimentally produced seizures the first finding, even prior to the appearance of interictal spike discharges, is the disappearance of the hyperpolarization usually observed in pyramidal cells after they fire (13). It ap-

pears that the inhibitory interneurons are normal and are themselves firing even at an accelerated rate; and there is no evidence that their efficacy in producing their usual conductance increases is impaired. The above evidence and the known effects of ammonia on the Cl^- pump make it attractive to consider the possibility that the hypothesis presented above explains the mechanism underlying the preictal increase in cellular activity and the ictal synchronous discharges.

Starting from an overactive focus in a given neural sector, there could be repercussions that would reverberate throughout the whole nervous system. Not only are the other sectors to which excitatory signals are being sent in danger of being pushed beyond their control limits by an excessive input, but also the excitatory presynaptic axons and terminals in the vicinity of overactive neurons may become hyperconductile in the presence of excess ammonia, which might act directly on the membranes as well as to diminish presynaptic inhibition exerted via transmitter liberated from axoaxonal connections from inhibitory interneurons. Thus, "during synchronous epileptiform discharges in neocortex, excitability increases of intracortical axons lead to repetitive spike electrogenesis near their terminal segment. Axon terminal bursting is directly associated with the development and spread of focal seizures in neocortex" (39).

At all times, one must consider the effects of various aspects of the stress response when seizures are considered. Probably with all changes of neural activity there may be an increase in the local release of some cybernenes in the CNS and in the peripheral release of hormones, a number of which may pass the blood-brain barrier and have effects in one or more brain regions. Generally, although the literature is confusing on many aspects and no consensus has been reached, it appears that as long as the through-put systems are still operating in a controlled fashion, the cybernene systems act in such a manner as to counteract a tendency of a neural region toward maladaptive hyperactivity. However,

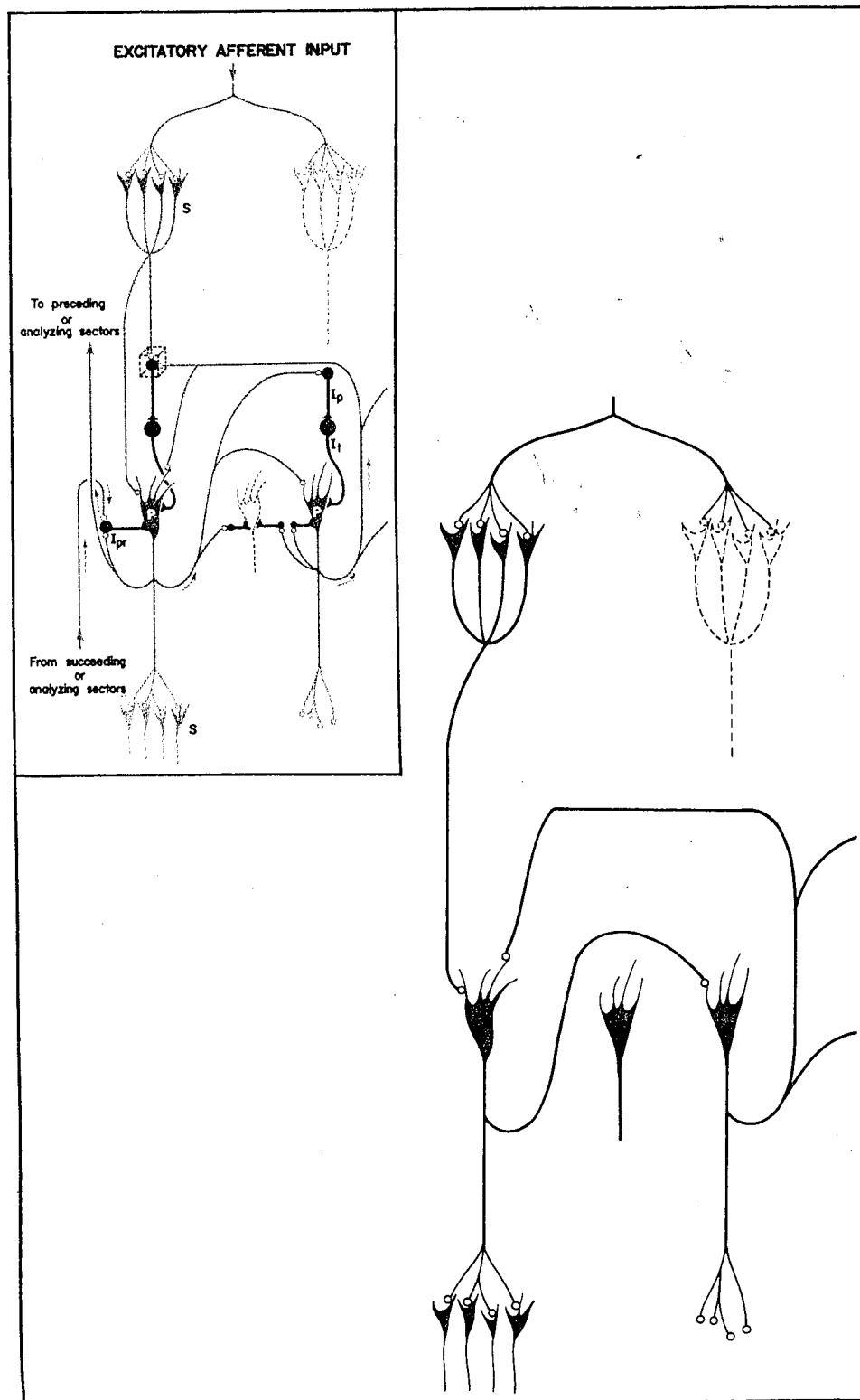


FIG. 9. Representation of a module or basic neural circuit (*see insert*) without inhibition. The inhibitory neurons may become ineffective because of failure to release inhibitory neurotransmitter, because of postsynaptic ineffectiveness of released transmitter, or for both reasons. This is meant to depict active neural circuitry during a paroxysmal discharge.

since the cybernene and hormonal systems probably have essentially the same type of neural control mechanisms as the through-put neural systems, an almost total loss of inhibitory control, as described above in seizures, would also occur in the case of the cybernenes and hormones. For example, if activity of a particular cortical region were to increase to a high level, one might expect the release of cybernenes (e.g., norepinephrine, peptides, dopamine, and serotonin centrally) and circulating hormones (e.g., epinephrine, ACTH, and steroids peripherally) to be adjusted in such a manner that less fuel would be added to the threatened fire—vasoconstriction would occur, oxygen and glucose availability would be decreased, cyclic nucleotide levels would be shifted in such a manner that membrane properties would be altered in the direction of decreased excitability, so that neural activity would tend to remain within the range required for the solution of the problems at hand. However, once a near-total loss of inhibition takes place, as in seizures, the release of a number of the above substances might take place in an unregulated and uncoordinated fashion. This could result in effects that act in the wrong direction, leading to an escalation in the excitability of neurons in the region at risk. There might be intensification and/or prolongation of seizures and the accentuation of their consequences by facilitating plastic changes among the neural elements active during the seizures that might lower seizure thresholds and increase the severity of subsequent seizure attacks. The interactions of the neural through-put and cybernene systems must be taken into account in all discussion of convulsant and anticonvulsant effects of drugs in intact animals. Although it is not possible to do so currently because of the paucity of suitable measurements, new strategies must be sought in which neurophysiologic and chemical measurements and specific pharmacologic manipulations can be used to give concurrent information relevant to such interactions.

SOME PERTINENT PHYSIOLOGIC AND PHARMACOLOGIC OBSERVATIONS IN NEURAL TISSUES

Numerous pharmacologic studies have been performed that have correlated changes in GABA levels and GAD activities with increases or decreases in seizure thresholds or the actual occurrence of seizures. An element missing in all but a few such studies has been the performance of pertinent physiological measurements showing that information processing in key neural regions was affected in such a way as to be correlated with the likely cause of the seizures. A particularly interesting earlier experiment was performed in cats employing the unanesthetized neuronally isolated cortex in which neuronal activity does not usually occur spontaneously but can be elicited by electrical stimulation (20). In this preparation, which is much more sensitive than normal cortex, possibly because of the loss of efficacy or decrease in numbers of inhibitory neurons, a single stimulus applied to the surface of the isolated cortex can elicit a burst of activity which spreads through the isolated cortical regions. In referring to Fig. 6, one can imagine that in the isolated cortical preparation prior to stimulation, the tonically inhibitory neurons (I_t) predominate and keep the P neurons inactive. The stimulus applied through a ball electrode for 1 msec released the activity of sufficient numbers of P neurons so that their activity, and the activity of P neurons recruited by them at sites away from the electrode, continued for several hundred milliseconds in the absence of further stimulus before inhibitory control was reinstated.

It was possible to distinguish the actions of two general classes of convulsant drugs applied directly to the cortex. Application of members of the first class, which included strychnine, picrotoxin, pentylenetetrazol, penicillin, *d*-tubocurarine, and thiamine, caused the almost immediate appearance of "convulsive" spikes in the activity elicited by a single stimulus. These abnormalities were indepen-

dent of the rate of stimulation and continued for the period of application of the drug. With the exception of thiamine, all of the substances listed above have been shown in one or several test systems to inhibit GABA-induced or glycine-induced increases in chloride conductance as one of their actions. Some or all of them also may increase the conductile properties of neural membranes. For example, penicillin has been found to produce selectively a nondepolarizing increase in the excitability of unmyelinated nerve endings. In other experiments, several convulsant hydrazides were applied to the cortex. In addition to other effects, the latter are known to be inhibitors of L-glutamate decarboxylase by virtue of their reactivity with its pyridoxal phosphate coenzymatic moiety and, therefore, inhibitors of the production of GABA. No effects were observed in the unstimulated preparation or during an initial period of 10 to 30 min of stimulation. Subsequently, spikes appeared during low-frequency stimulation, disappeared during continuous application of the drug when the stimulus was interrupted, and reappeared upon further stimulation. The faster the rate of stimulation, the more rapid was the reappearance of the convulsive spikes. A reasonable explanation of these results is that the convulsant action of the hydrazides might not be evident until the available stores of synaptically active GABA are depleted; and if the block of GAD were only a partial one, GABA would reaccumulate during a period in which no activity occurred.

The above experiments illustrate three ways by which enhanced predisposition to cortical seizures can be produced—a general increase in nerve membrane excitability, blockade of inhibitory (probably largely GABA) receptor mechanisms, and a decrease in the availability of the transmitter itself. The blockade of transmitter sites for inhibitory transmitter has virtually instantaneous effects which may be rapidly reversible, whereas the effects of a decrease in the rate of production of the transmitter are use dependent, and in the case of

carbonyl-trapping agents such as the hydrazides, are best reversible by administering some form of vitamin B₆ (pyridoxine, pyridoxal, or pyridoxamine) to replenish rapidly the pyridoxal phosphate required to reactivate GAD. There now are literally thousands of reports in the literature dealing with one or another variation of the latter two effects. They generally add up to the conclusion that the GABA system is extremely important in the vertebrate brain in helping maintain neuronal excitability below seizure threshold.

In another set of pertinent experiments, intracellular recordings were made in neurons in cat visual cortex (area 17) during the presentation of visual stimuli of optimal configuration before, during, and after the production of an epileptic focus by iontophoresis of penicillin (16). The first abnormality observed after penicillin application was an enhancement of previously observable responses to preferred stimuli, a result of an effect directly on the neurons themselves, on synapses made on them, or on both. This enhanced response could be elicited from a cell independently of the discharge activity of an induced focus, but only with stimuli appropriate for the receptive field. Subsequently, with further iontophoresis, high-frequency bursts of spikes either occurred spontaneously or were triggered by stimuli which were either effective or ineffective previously and were coincident with an extracellular paroxysmal depolarization shift. A model applicable to such altered interactions within a population of affected cells is given in Fig. 9.

MORPHOLOGIC OBSERVATIONS OF GABA NEURONS IN DISEASE

In this section the rather obvious, but important, case will be covered in which an actual destruction or loss of efficacy of inhibitory GABA interneurons has been proposed to take place. This is believed to be a major cerebral defect predisposing human beings to seizures resulting from traumatic injury, tumors,

interference with blood supply, or some hereditary metabolic defects. One of the first steps in determining whether or not GABA neurons are involved in a particular disease process should be to locate and study the GABA neurons and/or their processes in pertinent regions of the CNS at both the histologic and ultrastructural levels. Application of the currently available immunocytochemical tools to human material is difficult, and, except for biopsy samples, at the present time would probably have to be restricted to studies at the light microscopic level. The immunocytochemical technique for ultrastructural analysis for GAD requires fixation by rapid and adequate perfusion, a procedure that normally is not feasible in human material obtained several hours after death at autopsy. Furthermore, the techniques can be applied only to small, specific regions, since they do not lend themselves to broad screening approaches. It probably would be of some interest to study tissue with extensive pathologic changes, such as the striatum in Huntington's disease or the substantia nigra in advanced parkinsonism. On the other hand, it might be most informative to examine regions of the ventrobasal complex of the thalamus in Huntington's chorea, since, in the absence of grossly observable pathologic changes in this region, quantitative cytometric measurements showed there to be a loss of more than 50% of small Golgi type II neurons (presumably GABA neurons) in the ventrolateral thalamus by comparison with controls, and a decrease in size of those cells remaining (14). The latter type of neuron appeared unaffected in other thalamic sites. A loss of coordinative neural elements in the ventrolateral thalamus, a key relay station for processing information involved in motor control and muscle tone, could be importantly related to the choreic symptoms of the disease. Measurements on autopsy tissue obtained from schizophrenic and nonschizophrenic individuals have shown a statistically significant reduction in glutamic decarboxylase activity in the nucleus accumbens in schizophrenia (5) and decreases in GABA contents in the nu-

cleus accumbens and thalamus (41). Another study showed that of six brain regions measured, not including the nucleus accumbens or other limbic structures, only in the case of the thalamus were the values in the schizophrenic brains lower than those of the normal controls at the 5% level of significance (50). It is of considerable interest that, of the various thalamic regions studied, only the pulvinar showed a significant decrease (40%) in the number of Golgi type II neurons in the brains of catatonic schizophrenic patients in comparison with controls (14). Since the pulvinar plays an important role in coordinating visual and auditory inputs, the cellular defect observed, if confirmed, might be associated with the well-known hallucinatory phenomena observed in the above state. Is it possible that abnormal activity or actual bursts of seizure activity in the limbic system resulting from inadequate function of GABA neurons may be a link between epilepsy and psychosis (60)?

It has long been proposed that decreases in the efficacy of the GABA system could result in convulsive seizures, and much evidence has been adduced to support this belief. In order to test the above supposition directly at the cellular level, it is necessary to study the neuronal interrelationships in the CNS of organisms with various types of naturally occurring and experimentally induced seizures. It should be determined whether or not there are decreases in numbers of GABA neurons, whether their relationships to other neurons or to each other are disturbed morphologically, and whether or not the GABA neurons are impaired in their functional activity in such a way that the restraints on the activities of the excitatory pacemaker neurons in a given region of the CNS are weakened so that it has become easier for them to recruit each other into the runaway activities eventually observed in seizure states. The availability of immunocytochemical techniques and the identification of neuronal types as GABA neurons make it feasible to begin to attempt to answer some of the morphologic ques-

tions. As discussed in a preceding section, GABAergic neurons in the cortex have been identified by immunocytochemical procedures for visualizing GAD as aspinous and sparsely spinous stellate interneurons with extensive intracortical axonal arborizations forming numerous symmetric synapses. Immunocytochemical study of sections from sensorimotor cortex obtained from five electrographically proven epileptic monkeys treated with alumina cream made it possible to determine that a correlation existed between epileptic activity and effects of the treatment on GABA neurons (45). Mean numbers of GAD-positive terminals counted in contiguous areas from the bottom of layer VI to the middle of layer V showed, in each instance, a highly significant reduction in numbers of GAD-positive terminals at the electrographically epileptiform sites of alumina gel application by comparison with the contralateral nonepileptic homotopic cortex. Ipsilateral sections further away from the alumina gel also showed somewhat smaller but still highly significant decreases in numbers of visualizable GABAergic endings. These results support the idea that a relatively selective loss of inhibitory GABAergic neurons, the aspinous and sparsely spinous stellate cells, or the less likely loss of the GAD from GABAergic neurons, could be responsible for susceptibility to epileptic activity observed at seizure foci.

The latter observations are particularly pertinent since many epilepsies in human beings are caused by lesions in the brain resulting from trauma, such as head injuries, tumors, and cerebral angiomas. The above conditions may interfere with the normal blood supply to a particular region. GABAergic local circuit neurons, as well as other types (e.g., glycinergic), may be particularly susceptible to injury as a result of ischemic anoxia. This vulnerability also may extend to anoxia resulting from excessive functional demands and the large increases in metabolic demands and changes in physical state that may occur in febrile states. An extraordinarily heavy excitatory bombardment, whatever its source, to a

particular brain sector, might create conditions in the vicinity of the local circuit neurons, such as too great elevation of cyclic GMP level and depletion of glucose and oxygen and thus, ATP, in the affected region, which would adversely affect the function and possibly even the existence of the GABA interneurons in the region (29). This would sensitize the sector to epileptiform discharge.

Nature appears to have performed a pertinent human experiment in the form of the hereditary disorders known as the neuronal ceroid-lipofuscinoses, diseases in which a prominent feature is the occurrence of seizures. In general, it appears that these disorders are inherited in a mendelian recessive manner and that the various phenotypes are produced by different mutants (67). However, a dominant form of this class of disorders also has been described. It is interesting that in the dominant form of adult neuronal ceroid-lipofuscinosis, the first sign of the disease, in all instances, is the appearance of grand mal seizures (6).

The diagnosis of these disorders is based on the morphological demonstration of excessive amounts of autofluorescent lipopigments in neuronal perikarya, astrocytes, and certain visceral cells. These disorders are clearly chemically distinguishable from the gangliosidoses, since no accumulation of gangliosides is found, but their biochemical basis still is not known with certainty. Accelerated destruction of mitochondria and incomplete removal of lipid degradation products is one possibility. Some abnormalities may exist in the metabolism of the polyunsaturated fatty acids that result in the dialdehydic cross-linking of intracellular constituents into pigmented insoluble polymeric material. In preparations specifically employed for observation of pigment, but not in ordinary neurohistologic sections, it was determined in early stages of the disease that the lipopigment is stored in large amounts rather specifically in stellate neurons. Eventually, prior to more extensive damage, in such cases there seems to be selective destruction of the stellate cells in the cor-

tex, while the pyramidal neurons are preserved. Electron microscopic study of a cortical biopsy sample from 4-year-old girl with a moderately advanced stage of this disorder disclosed "an apparent rarity of type II synapses on the perikarya and axon hillocks of large projection neurons" (64). Types II symmetric synapses are typical of those formed by GABA cortical neurons. Type I synapses, typical of cortical excitatory neurons, remained abundant in the neuropil. It was suggested that such a differential loss of inhibitory synapses "might be the basis of the diffuse paroxysmal activity and other dimensions of cerebral dysfunction characteristic in this and similar cases." From the preceding discussion and the decrease in GABA content in the brains of patients with this disorder, it may be presumed that many of the cortical stellate cells that are destroyed are GABAergic neurons and that the progressive behavioral deterioration may be correlatable with the progressive loss of these neurons.

A synopsis of the clinical findings in this case is presented below so that the reader can get a feeling for the types of abnormalities in human behavior that possibly might be encountered with the progressive loss of GABA interneurons.

The patient walked at 11 months and talked in complete sentences and was toilet trained by the age of 2 years. Deterioration in her performance was recognized in the fall of 1974 at the age of 3 years. This was manifested by clumsiness, frequent falls, dysarthria, and loss of ability to speak in full sentences. Tremor and myoclonic jerking supervened and she was occasionally incontinent. In January, 1975, an EEG showed spikes, polyspikes, and atypical spike-wave complexes superimposed on a background of irregular high-voltage slow activity. At the age of 4 years, her gait was ataxic, and she was unable to walk without assistance. Her reflexes were hyperactive. There was no paresis, and the plantars were flexor. Cranial nerve findings were normal and there was no detectable somatosensory deficit. She seemed alert and followed one-step commands but spoke only in a few single poorly articulated words. Multiple EEGs obtained during the hospitalization showed poorly or-

ganized delta and theta activity with continuous polyspike activity. During the subsequent 9 months of observation, she became increasingly irritable and prone to temper tantrums. She spoke and followed simple verbal commands less consistently. Her tremors increased in amplitude and became more generalized. Single myoclonic jerks interrupted distal extremity movements and there were brief lapses of postural tone. Muscle tone and tendon reflex activity increased and became associated with ankle clonus, but the plantars remained flexor. She could no longer stand alone or walk with assistance at the time that the cerebral biopsy was performed.

In most of the other cases described in the literature, in addition to findings similar to those above, grand mal seizures have been a prominent feature of the symptom complex.

ANTIEPILEPTIC DRUGS AND MECHANISMS OF THEIR ACTION

In the study of any pathologic phenomenon, one aim is to identify the rate-limiting steps, bottlenecks, so to speak, at which the tools at hand may enable one to choke off the spread of the manifestations of the pathologic state. Often the best strategy is to try to focus on key events as close to the origin of the problem as possible because the consequential, ever-widening ripples at every point of advance of the pathologic process create subsidiary problems that often are unpredictable and may eventually require additional therapies far removed from the original problem. There are multiple causations and many overt manifestations of epileptiform phenomena. What do all of them have in common? The most apparent common denominator and the hallmark of seizures is that principal neurons, which normally are involved largely as individuals or in small groups in highly specific aspects of information processing in a given neural sector, first begin to fire abnormally frequently when engaged in performing their regular assignments and then join other neurons in the same sector in a series of relatively simultaneous impulses at high frequency in a manner irrelevant to their role in information process-

ing. Eventually this may lead to self-sustaining discharges in adjacent and even distant neural sectors with various behavioral consequences occurring.

The most salient feature that emerges is that in seizures some neurons are firing with maladaptively high frequencies. The firing mechanisms probably are similar in most neurons that show an action potential. It would appear that if a substance could be administered or a procedure performed that could serve as a low-pass frequency filter with a cutoff point that would still allow neurons to perform their normal functions at the lower frequencies of firing ordinarily required, one might affect events close to the source of origin of seizures. Furthermore, it is possible that such an approach may also give differential effects on through-put principal neurons and on inhibitory interneurons. At least some of the latter may not use action potentials but may release transmitter as a continuous function of membrane potentials that fluctuate within the release portion of their input-output curves and might maintain transmitter release indefinitely (21). These nonspiking interneurons might be much more resistant to procedures with a relatively specific effect on the spike-generating mechanism. The finding that a number of local anesthetics possess anticonvulsant properties at low levels but are convulsants at higher concentrations suggests that these substances may exert such differential effects (see discussion below). Nature's own frequency filters probably reside at inhibitory synapses. Regionally liberated cybernetes and possibly some circulating factors also probably affect the functions of inhibitory synapses.

Another general approach to decreasing neuronal excitability would be to increase conductances of neuronal membranes of K^+ and/or Cl^- , which could produce hyperpolarization and shunting currents that would decrease the tendency of cells to fire. GABA and GABA-mimetics produce hyperpolarization via the Cl^- mechanism, as discussed in preceding sections of this chapter. Recently, valproic acid has been shown to produce an

increase in membrane conductance to K^+ , resulting in hyperpolarization of neurons in *Aplysia* (62). More depolarization is required to cause a given cell to fire when the conductances of the above ions are high than when they are low. A third possibility lies in influencing synaptic processes in such a manner that liberation and postsynaptic efficacy of excitatory transmitters generally would be decreased and/or that of inhibitory transmitters increased. The last item to be added to the potential armamentarium would deal with the prevention of initiation of seizures in an overactive nervous system by preventing it from going "critical," that is, by preventing the occurrence of an event, such as a local increase in ammonia level might be, which converts a cybernetic system to one in which excitatory events can take place. Let us now examine the known antiepileptic procedures and drugs from the above points of view.

Several Substances with Anticonvulsant Action Also Produce Frequency-Dependent Nerve Block⁸

As mentioned above, perhaps the most general approach that might be applicable to the treatment and/or prevention of epileptiform discharges in the nervous system would be to affect the conductile properties of those nerves that generate action potentials in such a way as to set a ceiling on the frequencies with which they could fire, while having little or no effect on inhibitory interneurons, many of which may not have a spike-generating mechanism. It has been known for some time that local anesthetics exert their action by producing a conduction block in peripheral nerves by blocking transmembrane sodium current (10). In addition, quite aside from knowledge of their mechanisms of action, local anesthetics have been used to prevent

⁸ The author is indebted to Dr. K. R. Courtney, Palo Alto Medical Research Foundation, Palo Alto, Calif., for guidance in the preparation of this section, for making unpublished information available, and for furnishing Fig. 10.

or abort a variety of seizures in animals and in man, suppressing spike generation in cortical epileptic foci and even afterdischarges in electrically stimulated isolated brain slabs (3). Recently it has been found that the nerve blocking action of local anesthetics is frequency selective (12). An example of such action is illustrated in Fig. 10. An untreated desheathed frog sciatic nerve can follow a 40 Hz stimulus without spike attenuation (Fig. 10A). Figure 10B shows that mepivacaine, a local anesthetic, at a concentration of 1 mM, attenuated the compound action potential only slightly when the nerve was stimulated infrequently, but that almost all excitability was blocked when the nerve was stimulated at a rate of 40/sec. A similar result was obtained when the experiment was performed in the presence of an even lower concentration of phenytoin (0.074 mM) (Fig. 10D). Similarly, phenobarbital was found to produce a frequency-dependent nerve block in this preparation (26).

The differential blockade by local anesthetics of sensory impulses in preference to motor impulses possibly may be attributable to the fact that neurons bringing peripheral signals

into the CNS (e.g., pain signals) may often fire with higher frequencies than those dealing with motor functions. Frequency-dependent conduction block may play an important role in the analgesia associated with the actions of local anesthetics. However, without actual measurement, little can be inferred about specific central actions from studies of peripheral nerves, since frequencies of firing of neurons in circuits released in the CNS subsequent to primary afferent activation may be very different from those of their inputs.

Frequency-dependent nerve block by local anesthetics has been shown in voltage clamp experiments to be attributable to a blockade of the sodium channels responsible for generation of the action potential (23). There appears to be a preferential, reversible binding of these drugs to some structural component of open sodium channels; and, therefore, the efficacy of blockade of nerve excitability by these substances is greatly enhanced when a nerve is firing rapidly in comparison with that observed when the frequency of firing is low. The degree of block depends on the rate of opening of sodium channels (11). Substances that exert such effects also have been found

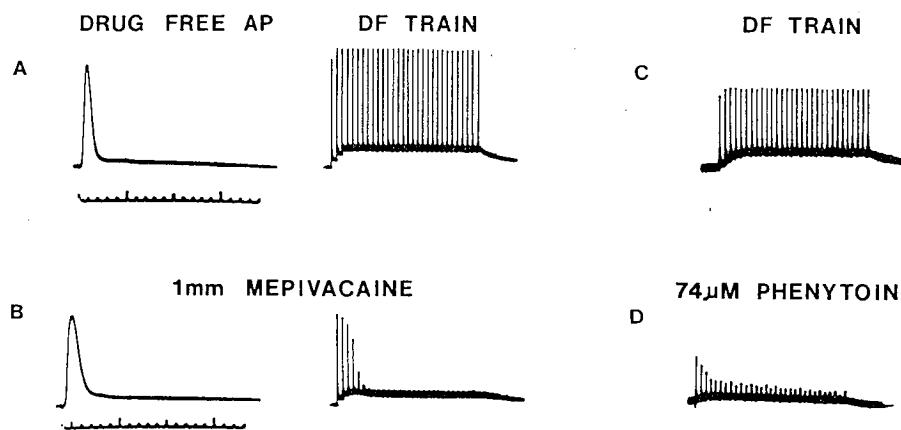


FIG. 10. Frequency-dependent nerve block by a local anesthetic and anticonvulsant. **A left:** Compound action potential recorded from desheathed frog sciatic nerve preparation. **A right:** 40 Hz train of stimulated action potentials photographed on much slower time base, showing that drug-free preparation can follow a 40 Hz stimulus without spike attenuation. **B:** Similar set of records to those in A but with local anesthetic treatment producing frequency-dependent conduction block. **C:** 40 Hz train record in another drug-free preparation. **D:** frequency-dependent conduction block produced by treatment with phenytoin. Time calibration: 1 msec/division on leftmost traces and 50 msec/division on other (train) records. Spike amplitude: about 30 mV recorded across a sucrose gap. (Figure kindly furnished by Dr. K. R. Courtney.)

to have antiarrhythmic pharmacologic effects. Phenytoin has widespread therapeutic use as an antiarrhythmic drug as well as an anticonvulsant (24). It can block dangerous high-frequency myocardial activity through a frequency-selective effect that is similar to that on nerve. Several antiarrhythmic drugs (24), including propranolol, which also has β -blocking and antipsychotic effects (51), have been shown to block myocardial excitability in a frequency-dependent manner, an action probably contributing importantly to their antifibrillatory efficacy.

It is apparent from a host of contributions, including those in this volume, that both convulsant and anticonvulsant drugs can affect the conductile properties of neurons at dendritic, somal, axonal, and terminal levels and also can affect the receptive properties of post-synaptic sites. It seems highly unlikely that the most widely used convulsants or anticonvulsants exert their actions only at particular specific membrane receptor sites.

Some Structure-Activity Considerations

The above similar actions of such diverse substances as local anesthetics, propranolol, phenobarbital, and phenytoin suggest that the interactions of drugs with sodium channels of the "modulated receptor" type (23) may be more important than hitherto suspected in trying to interpret their effects on excitable membranes. Such mechanisms may play an important role in the action of antiepileptic drugs. The structural differences in the above substances and experimental results suggest that local anesthetics probably act at different molecular sites of the sodium channel apparatus than phenytoin and phenobarbital. At the molecular level, it is possible that local anesthetics may associate primarily with anionic sites on the polar head groups of membrane phospholipids. Phenytoin, barbiturates, and similar substances may interact chiefly by hydrogen bonding with the CO and NH groups of peptidic bonds of unfolded β -chain

portions of membrane structural proteins (as suggested for phenytoin in Chapter 12) and with the hydrocarbon portions of the fatty acid side chains of membrane phospholipids. The latter suggestions are in keeping with the findings that local anesthetics and barbiturates may act synergistically in preventing cortical afterdischarges in monkeys (3). The observation that calcium binding to phospholipids can be inhibited by procaine and stimulated by phenobarbital (Chapter 18) suggests that the interaction of the barbiturates with membrane proteins may displace phospholipids from interaction with some portions of membrane proteins in such a manner that the anionic groups on the phosphate moieties become available for binding with Ca^{2+} or the cationic groups of local anesthetics. However, in general, it is extremely difficult to deal meaningfully with structure-activity relationships among neuroactive drugs because of almost total ignorance of the molecular details of neural membrane structure. The structures and conformations of most membrane proteins are not known, and the nature of the relationships between lipids and proteins in neural membranes still is obscure.

Approaches to seeking structure-activity relationships in the field of anticonvulsant drugs have ranged from sophisticated statistical analyses of chemical data and anticonvulsant potency in available test systems (Chapter 14), to attempted correlation of convulsant and anticonvulsant activities of substances with theoretically derived all-protein receptor sites based largely on variants of the protein β -chain (Chapter 12), and to detailed stereochemical structural analysis of antiepileptic substances (Chapter 13). At present, the above approaches have not yet led to major advances in rapid and specific design of antiepileptic drugs.

Inspection of molecular models of a single protein β -chain in the vicinity of a L-glutamate residue and phenytoin indicated that hydrogen bonding could take place between the CO and NH groups of phenytoin with suitably

spaced NH and CO groups, respectively, of the β -chain and with the COOH group of the glutamate residue. Likewise, there are a variety of hydrogen-bonding possibilities of the above protein groups with barbiturates, *N*-desmethyl diazepam, desmethylsuximide, prostaglandin E₂, 5,5 dimethyl-2,4-oxazolidinedione, β -hydroxybutyrate, and acetoacetate. Such a formulation suggests that a necessary, but not sufficient, condition for anticonvulsant action may be the ability of the above classes of antiepileptic substances to hydrogen-bond with extended peptide chains in the β -configuration and with some amino acid side chains. Of course, the active substances may have to possess lipid solubility to be able to penetrate to such sites and become firmly anchored to them. Although the hydrogen bonding properties mentioned above may seem too general, it is the only unifying chemical hypothesis that we currently can advance to account for the anticonvulsant actions of such a diverse group of substances. It is almost certain that the major effects cannot be exerted solely on specific neurotransmitter receptor sites, because the latter generally appear to be too fastidious in their molecular requirements. Undoubtedly, membranes in specialized regions of neurons probably share structural components, some of which may be protein β -chains. Substances that combine with components of these β -chains may alter their relations to other protein units and/or to membrane lipids. It, therefore, makes sense that some of the substances referred to above might affect conductile properties of dendrites and axons, storage and release of transmitters from presynaptic endings, reactivities of ion channels to transmitter impingement at postsynaptic sites, activities of membrane-bound enzymes, and the degrees of carboxymethylation and phosphorylation in the regions of the membrane to which they bind, and, therefore, the affinities of membranes for cations. Most of such effects in one or another test system have been described for phenytoin and a number for bar-

biturates in the chapters devoted to these drugs.

The reader may be surprised to find the ketone bodies, acetoacetate and β -hydroxybutyrate, included in the above list of substances. One of the oldest effective treatments for epilepsy is the ketogenic diet (Chapter 41). Of all the biochemical variables evaluated to date in animals and people receiving such diets, the only consistent finding is that there is a reasonably good correlation of plasma levels of the ketone bodies with the anticonvulsant effects of the diet. It has been conjectured that "one or both of these compounds either have direct anticonvulsant effects or produce rapidly reversible changes in cerebral metabolism, which in turn affect cerebral excitability" (25). Although the first of these suggestions is an attractive one, we could find no record of the administration of the above substances individually or together in experimental tests for anticonvulsant action. In view of the potentialities of these substances for hydrogen bonding with structural proteins in nerves in a manner similar to known anticonvulsants, tests of their anticonvulsant potency in animals and in a variety of neurophysiological test systems would seem to be in order. Both acetoacetate and β -hydroxybutyrate are metabolized rapidly and would be likely to be removed particularly rapidly in tests in animals in which normal dietary levels of carbohydrate are employed (Chapter 41). This handicap might be circumvented by the use of animals on a high-fat, low-carbohydrate diet or, alternatively, by examining for anticonvulsant activity less readily metabolizable β -keto or β -hydroxy carboxylic acids with structures that allow them to form hydrogen bonds in the same manner as acetoacetate and β -hydroxybutyrate. Two possibilities that suggest themselves immediately are 2-*n*-propyl-3-hydroxypentanoic and 2-*n*-propyl-3-oxopentanoic acids, recently discovered metabolites of valproic acid (31). The mechanism of the action of the latter effective anticonvulsant agent still is not established (Chapter 42). There are co-

gent reasons for questioning the hypothesis that valproic acid exerts its action solely via the inhibition of GABA transaminase and the consequent accumulation of GABA (56). None of the metabolites of valproate, including the two mentioned above, has yet been reported to have been tested for anticonvulsant activity or examined in any neurophysiologic test systems. In any event, a large number of β -keto and β -hydroxy carboxylic acids either already are available or can be synthesized without difficulty for such testing.

Prostaglandin E₂, but not F_{2 α} , can prevent seizures induced by pentamethylenetetrazole in rats and tremors induced by harmaline in cats and rats (40). Both prostaglandin E₂ and methyl analogues of it may prove to be highly effective anticonvulsants under some circumstances (4). Models of the prostaglandin E₂ and F_{2 α} showed that only E₂ could readily form hydrogen bonds with an extended polypeptide β -chain. Here again, the study of prostaglandins at the membrane level is just beginning, and detailed assessment of their general potential utility in treatment of seizure disorders is awaited.

DEVISAL OF NEW ANTIPILEPTIC DRUGS

In any field of medicine, it is a blessing when there are test systems that have a one-to-one correspondence with a therapeutic goal to be achieved and when the molecular basis of a defective function is sufficiently well known so that rational structural variations can be made in potentially useful therapeutic agents with a view to optimizing their efficacy. This is not the case for the epilepsies. A number of systems in whole animal have been employed generally for the screening of potential antiepileptic agents, and now to these have been added several comminuted test systems such as neurons in tissue cultures and in the neural subsystems of various invertebrate preparations, such as the crayfish stretch re-

ceptor. The expansion of the armamentarium of physiologic and pharmacologic techniques currently available will not necessarily be helpful in the understanding of the mechanisms of the epilepsies or in the targeting of synthesis programs for specific agents to control them. What seem to be missing are test systems that can be identified with specific aspects of neural function that are rate limiting in seizure phenomena. For example, in a preceding section it was mentioned that a number of substances with anticonvulsant action also have been found to produce frequency-dependent nerve block. I would like to suggest that systems in which it is possible to study such effects should be added to the screens currently employed and that structure-activity relations of the members of the classes of available substances of varying degrees of anticonvulsant potencies be examined. If a good correlation were found between clinical efficacy and potency in the above test systems, it should be possible to further simplify and quantitate the systems for rapid screening of potentially useful drugs and for studies of mechanisms of action. Frequency-dependent nerve block by local anesthetics and their anticonvulsant effects probably are related to a blockade of sodium channels that are responsible for the generation of the action potential (11). There are many possibilities now of creating artificial membranes that may possess conductile properties similar to those of nerve membranes and of applying various physical and chemical measurements to such simplified systems. Perhaps eventually it will be possible to constitute standard responsive artificial membranes with suitable combinations of macrolides, channel proteins, and phospholipids. There is no lack of general and relatively unfocused biological screens for testing antiepileptic substances. Rather, there has been a paucity of specific hypotheses that could be tested quantitatively by relatively simple procedures in readily analyzable systems.

Great emphasis has been placed on the role of GABA neurons in normal and abnormal

activities of central nervous systems. In a nervous system that has been proven to be susceptible to seizures, consideration should be given to strengthening the effectiveness of the GABA system as much as possible in order to decrease the possibility of recurrence of seizures. An approach to the correction of an imbalance between the excitatory and inhibitory systems, in which the GABA system may be a weak component, might be to attempt to devise methods by which the potencies of GABA neurons could be enhanced. A promising new direction has been the recent development of irreversible specific enzyme inhibitors of GABA transaminase, which elevate GABA levels throughout the brain and possibly at sites of presynaptic release. Several substances are now available which are acted on by the latter enzyme to produce an intermediate that combines covalently with the enzyme and inactivates it permanently (35,36,57,58). These agents have been shown to greatly increase the GABA contents of the brains of experimental animals and concurrently to protect them against a variety of seizures. Thus mice have been protected against electroshock or audiogenic seizures by the administration of such substances, and photosensitive baboons have been protected against myoclonic or epileptic seizures on exposure to light. Currently this type of manipulation looks more promising than other possible modes of enhancing efficacy of GABA neurons mentioned in preceding sections of this chapter.

If my hypothesis about the role of ammonia in causing the transition from an excitable to an epileptic focus is correct, approaches may be sought by which liberation of ammonia by neural elements during excitation can be decreased or extracellular levels of ammonia can be kept at an extremely low level. In this connection, it is of considerable interest that arginine, a substance that protects animals against the lethal effects of administered ammonia (27), also protects animals against hydrazine toxicity (15). Both hydrazine and ammonia may, in part, exert their deleterious effects on

nervous system function by inactivating chloride channels (28,30,37,42,54). Arginine is believed to exert at least part of its protective action in ammonia toxicity by accelerating the rate of removal of ammonia by conversion to urea in the animal organism (38). It would be of interest to determine whether administration of arginine, or other types of procedures by which ammonia contents of extracellular fluids can be reduced, might be an adjunct in the therapy for epileptic disorders.

It is difficult to devise logical therapies for conditions in which the normal functions of the tissues are not clearly delineated. This volume presents in great detail many aspects of the action of anticonvulsant drugs and subjects related to these actions. One must keep an open mind, remembering that most of our useful anticonvulsant drugs were discovered serendipitously, often during empiric experiments. On the other hand, one's scientific predilection is to get as close as possible to the cellular and molecular sites of action. It is largely for this reason that I have tried to furnish a framework that may allow the reader to organize some of these facts sufficiently so that useful patterns will emerge and, therefore, catalyze new experimental and theoretical approaches to the devising of agents that would have maximal anticonvulsant potency with minimal side effects.

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