EFFECTS OF DRUGS ON THE CENTRAL NERVOUS SYSTEM[1]

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The editors instructed me to be "fiercely selective" in the material that I was to include in this chapter. In one respect that is an easily followed instruction for a general physiologist who studies cell membranes and attempts to use pharmacology as a tool to help decipher the electrogenic phenomena of excitable cells. Electrophysiology is fortunate in having a theory which links electrogenesis with the permeability of the cell membrane to ions (25, 100). The dazzlingly quantitative application of this theory to account for the spike of the squid axon (101) is meeting with the stubborn complexities of living membranes (13, 14, 95, 144, 188, 189). Nevertheless, some relatively simple modifications of the ionic theory permit the latter to account, although only qualitatively at present, for the bulk of a large variety of electrophysiological and pharmacological phenomena (84, 85, 89, 90).

The situation appears to be quite different in pharmacology. The periodical literature seems to be styled after the biblical "begat" chapters and much of it is about equally innocent of theory. Even in the restricted field of neuropharmacology the data are almost entirely based on various reactions of whole animals or of their individual organ systems. When theory is attempted it seems to regard as irrelevant the morphology and functional properties of individual neurons and effectors, and of their organization into the complex organ system of the neuraxis and its peripheral appendages.

In another respect, however, this instruction must be disobeyed. The present reviewer arrived at his interest in neuropharmacology from an attempt to use a relatively simple conceptual framework, gained from the study of peripheral junctions (78 to 80), to account for the actions of drugs on cells, including those of the central nervous system (77, 81). The studies on electrocortical phenomenon with Purpura and other colleagues (82, 148, 151, 152, 154 to 156) in which pharmacological tools were used extensively

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This chapter is dedicated to the memory of my friend and colleague Abraham M. Shanes, whose debut as a professional pharmacologist was initiated by a work (179) which will long remain a classic in the field.

showed that these concepts could yield an internally consistent explanation of many old and new pharmacological as well as electrophysiological data. Thus, in attempting to evaluate some recent papers on pharmacology of the central nervous system the reviewer will continue to call on data from simpler peripheral systems, and even from invertebrate material.

The inclusion of data on simpler neuronal systems can now be quite readily justified. Numerous studies have been made in the past years with intracellular recordings from neurons in the mammalian central nervous system. Among those of the current period may be cited references (3, 40, 76, 111, 129, 134, 150, 158, 159, 161, 172, 203). These data substantiate the view that neurons of the central nervous system in vertebrates have essentially the same basic properties as do neurons of the spinal cord (53) or various peripheral junctional systems (85). Cortical neurons of mammals develop excitatory and inhibitory postsynaptic potentials (e.p.s.p.'s and i.p.s.p.'s respectively) and in various details of their electrophysiology behave as do neurons of simpler systems. The data are complicated not so much, then, by special properties of neurons as cells, but by the fact that vast numbers of cells are intricately interlocked functionally.

Of necessity many topics will be omitted. Current or still useful references to summaries of the literature on these topics will be found in the following: (34, smooth muscle); (36, alkali-earth ions); (51, nonmyelinated fibers); (53 and 54, synaptic transmission and chemical mediators); (66 and 67, synaptic transmitters); (110, reticular formation); (114, cholinesterase and anticholinesterases); (146, olfactory system); (173, drugs and membrane barriers); (178, serotonin and catecholamines); (181, action of nicotine on central nervous system); (195, drugs and cardiac activity); (196, enzymes and transmitters); (201, carrier transport); (205, olfaction and taste).

Theories in Neuropharmacology

At least in the United States, the dominant view on the action of drugs appears to be heavily in favor of biochemical explanations, such as interference with enzyme systems or with the biochemical ecology of the organism. These views are discussed with sympathy, but with admirable restraint, in previous volumes (33, 204). Such restraint is greatly needed, since speculations far outrun the data. Attempts which have been made to correlate biochemical findings with relatively well defined physiological conditions (23, 24, 132) disclose that there is considerable discrepancy between biochemical and electrophysiological changes.

This reviewer would take issue with the statement (204) that the study of two groups of "target enzymes" (cholinesterase and monoamine oxidase) has "contributed a better insight into the mode of action of certain drugs." This is certainly not the case for the actions of some anticholinesterases. Physostigmine can block synaptic transmission in eel electroplaques about as effectively as does *d*-tubocurarine (1). Other anticholinesterases (neostigmine, DFP) do not, but depolarize the cells, and thus are cholinomimetics. In the

rat auricle also, DFP is a cholinomimetic, and its action is blocked by atropine, whereas physostigmine is a competitive antagonist of acetylcholine (16). Physostigmine is also a blockader of transmission in frog sympathetic ganglia (27). Procaine, which is another curariform agent in eel electroplaques (1), is antagonized by acetylcholine in its effects on frog axons (29). The output of salivary glands is progressively increased by more DFP (164). This is a strange behavior for a blockader of enzymatic activity, but can be explained in terms of a general electrophysiological theory (77, p. 563). The anticholinesterases improve the effectiveness of labile cholinomimetics or acetylcholine, but they are also synaptic agents, some being themselves cholinomimetics, others competitive antagonists of acetylcholine. Reversals of adrenergic activity, which will be discussed below, would probably also offer difficulties for biochemical theories.

The classical explanations of the action of drugs, as due to reactions between the latter and receptor sites on the cell membrane, would appear to be the more realistic approach, given the present-day evidence for the importance of electrogenesis in the activity of neurons. Following Clark (41) and Schild (174), several workers have developed mathematical relations based on the law of mass action and of reactions between receptors and drugs. A two-factor theory has been proposed independently by Ariëns (6, 7) and by Stephenson (183) and has been developed extensively by Ariëns and his colleagues. According to their view, the drugs must be specified not only with respect to affinity for the substrate, but also with respect to "intrinsic activity" or "efficacy." Paton (147) has taken a somewhat different approach to include a second parameter, specifying that pharmacological activity is dependent upon the rate of interaction between receptor and drug.

It is noteworthy, and perhaps reflects the biochemical bias of American pharmacologists, that the large number of recent papers from the Nijmegen group (8, 9, 165–169 and others cited in these) have hardly been mentioned in previous volumes of this series. None were cited in the last volume. The techniques and vast amount of data developed by this group deserve closer study. The use of two parameters provides a built-in assurance that more data can be ordered and classified than would be possible by employing only the one factor of chemical affinity. However, it seems to this reviewer that the work is flawed by an abstract approach to the specifics of the various types of material to which the drugs are applied. Much of the work has been done on various smooth muscle preparations in which it is likely (cf. 77) that excitatory and inhibitory synapses interact. Indeed, the presence of both types of synapses has been shown in fibers of dog retractor penis muscle (145). The excitatory synapses have an adrenoceptive membrane, and the inhibitory membrane is cholinoceptive.

Whenever antagonistic physiological processes are involved, the resultant overt effects of drugs become unclear. A classical example is the characterization of strychnine as a "stimulant of the central nervous system" whereas its fundamental action is to block inhibitory synapses (cf. 42, 43). It may be ex-

pected a priori that the actions of competitive antagonists will be relatively simple, and the data on such substances are orderly in the work of the Nijmegen group. However, the data on "noncompetitive" antagonists become quite complex, and van Rossum (166) has attempted to offer a "molecular" explanation for the complexities. It nevertheless seems probable that these complexities may reside in the heterogeneity of the synaptic organizations of the test objects. Furthermore, even in the absence of inhibitory synapses, when the preparations used are skeletal muscles or autonomic ganglia, the systems are heterogeneous. The synaptic and conductile membrane components in general react differently to drugs and these differences can alter the interactions which are essential for normal functioning of the cells. Depolarizing blockage of the conductile component has led textbook writers to classify succinylcholine as a "curarizing" drug merely because of the overt manifestation of neuromuscular paralysis, and K is usually also classified as a "depolarizing" and "curarizing" agent, although it probably does not affect the responsiveness of synaptic membranes.

Manifestations of complexity in autonomic systems are numerous. The reversals of activity in adrenergic systems by adrenolytic agents are well known (71) and recently have been studied further (133, 140). Still more complex are reactions like that of vascular smooth muscle to hydralazine (128, Fig. 63). After administration of this adrenolytic agent, epinephrine causes vasodilation, whereas norepinephrine continues to be a vasoconstrictor.

Toman (194) has summarized the attempt of the present reviewer to interpret the actions of drugs in terms of their effects on electrogenic mechanisms (77, 81, 85). A brief restatement of these views will be included partly because Toman's version was wrong in a few respects and partly to stress at this time that effects on nonsynaptic electrogenic membrane must be included in the analysis of drug action. At the time the electrophysiological theory of drug action was proposed, data on nonsynaptic effects were scant, although some effects were already known (cf. 78, 80). In the past few years, however, more data have become available on the effects of drugs on the output membrane of the nerve terminals (85, 96, 127, 163), as well as on conductile membrane in various types of cells. The review and theoretical analysis by Shanes (179) and his chapter in the previous volume (180) provide much information on the pharmacology of conductile membranes.

In the electrophysiological theory, the effects of drugs are regarded as being mediated through their modification of various elements of electrogenic activity of cell membranes. Receptive and postsynaptic membranes which are electrically inexcitable must of necessity respond to specific stimuli, and in the case of the synaptic elements the latter are most probably chemical agents (transmitters).[3] Presumably for this reason, many synapses are par-

[3] The reviewer may be accused of excessive caution with respect to the chemical theory of transmission. However, the reserve is not so much in regard to the theory, as to its uncritical acceptance. It is perhaps excusable for an elementary textbook

ticularly susceptible to other chemical agents. However, the veratrum alkaloids are classical examples of drugs which affect the electrically excitable conductile membrane. The marine "clam poison," tetrodotoxin (references in 141), and tarichatoxin (104) provide other examples. Furukuwa et al. (74) and Cheymol et al. (38) had indicated that tetrodotoxin appeared to block presynaptic activity. After neuromuscular blockage was set up, the end plate was still depolarized by acetylcholine (74). The data available did not then permit decision as to whether or not the output of transmitter was also affected. However, the "clam poison" blocks neuromuscular transmission in lobster without appearing to affect the size or the frequency of spontaneous "miniature" postsynaptic potentials (p.s.p.'s) (96). Nakajima et al. (142) have recently shown that the electrically excitable process of Na activation is blocked by tetrodotoxin in frog muscle fibers. Spike production is accordingly blocked, but the process of K activation which causes delayed rectification of the conductile membrane remains essentially unaffected.

Complementary data have recently also been obtained on a number of sensory neurons. The application of tetrodotoxin does not affect production of generator potentials in crayfish stretch receptors (132, 143), Pacinian corpuscles (141), *Limulus* photoreceptors (19), or insect chemoreceptors (202), The application of tetrodotoxin eliminates spike electrogenesis in all of these. Delayed rectification, which is indicative of K activation, is still present and block of spike production is presumably achieved by the elimination of Na activation. The action of the toxin is localized to the site of application of the agent in insect chemoreceptors.

The foregoing analysis of the action of tetrodotoxin points up the likelihood (77, 81) that commonly accepted pharmacological classifications may be quite superficial and even erroneous. Tetrodotoxin has been termed a "noncompetitive, nondepolarizing" agent for eel electroplaques (99). Presumably, the term "noncompetitive" is with reference to acetylcholine. The toxin blocks electrical activity of the electroplaques without depolarization, and even in very high concentration it does not prevent depolarization by acetylcholine, nor does it diminish the sensitivity of the electroplaque to the latter agent. The tetrodotoxin probably acts on the electrically excitable membranes of both axons and electroplaques, thereby preventing both indirectly and directly evoked responses. As in the other tissues cited, it does not affect the responsiveness of the electrically inexcitable membrane, which continues to react to acetylcholine.

These new data reinforce an earlier conclusion (84/ 85) that depolarizing electrically inexcitable and electrically excitable membranes have fundamentally different types of linkage of their electrogenic processes. Na activa-

(136) to employ the term "chemical synapses." However, in most cases of well-studied synaptic systems, the direct evidence that the transmission is mediated by chemical agents is simply absent (85, 92, 193, 194). Indeed, the role of acetylcholine in various cholinergic systems is still unsettled (114).

tion and K activation can be readily uncoupled in the electrically excitable type of membrane, but thus far it has proved impossible to separate the two processes in synaptic or receptive membrane. The difference may be of considerable theoretical importance when it becomes possible to deal realistically with the molecular structures of electrogenic membranes.

Also theoretically important are recent findings of the occurrence of electrically excitable Cl activation (20, 39, 93), and the demonstration that delayed rectification in frog slow muscle fibers is due to K activation (18). Thus, the electrically excitable membranes can respond with repolarizing electrogenesis as does membrane of inhibitory synapses. Pharmacological block of electrically excitable K or Cl activation can be produced by alkali-earth ions, particularly Ba.

The Cl activation evoked in Rajid electroplaques by depolarizing stimuli may be regarded as an "excitatory" activity. The resistance of the electroplaques falls. Thus, a larger proportion of the current generated by the cell during the normal neurally evoked response [which is a p.s.p. of electrically inexcitable membrane, as in other marine electric fishes (94)] is available to the external medium (20). The electrically excitable K activation of slow muscle fibers, on the other hand, is "inhibitory" since it reduces the postsynaptic depolarization of the neurally evoked activity and thereby probably reduces the coupling between depolarization and contraction (84). Thus, the number of varieties of actions of drugs on nonsynaptic membranes is also large and they may elicit various types of "excitatory" and "inhibitory" effects in the overt responses of the cells.

It now seems clearly established that the inhibitory synapses of cat motoneurons operate through the mechanism of Cl activation. Properly speaking, the inhibitory membrane is anion-permselective and the Canberra group (4, 5, 103) has carried out an extensive study on the permeability of the inhibitory membrane to various anions. It is claimed that only anions which when hydrated are no more than 1.14 times the diameter of the hydrated K ion can penetrate the activated membrane. However, calculations of ion diameters are subject to considerable error and one may question the degree of significance of the cited figure.

Cl activation is also the mode of activity of inhibitory membranes in neurons of *Aplysia* (191, 192), and *Helix* (106, 107). The inhibitory synapses of crayfish stretch receptor also operate through Cl activation (98), as do the neuromuscular inhibitory synapses of crayfish and lobster (30, 97). The inhibitory synapses of the vertebrate heart which are activated by vagal stimulation now appear to be the only known example of postsynaptic membrane which operates by K activation (102, 195).

The depolarizing electrogenesis of excitatory synaptic membrane was at one time regarded as due to a general increase in permeability of the membrane (35). However, it now seems likely that the responses are caused because of increased permeability only for Na and K (85). The best evidence thus far on this score is that on frog end plates (185 to 187). Thus, the elec-

trogenic processes of electrically excitable and electrically inexcitable membranes are essentially symmetrical (84, 90). One difference, which may be inherent in the different modes of their excitability, is that the electrically excitable membrane may react also to hyperpolarizing stimuli. Another, already mentioned, is the relative independence of Na and K activation in the electrically excitable, but apparently not in the electrically inexcitable depolarizing membranes.

The properties of the presynaptic nerve terminals are relatively little known chiefly because of the difficulty of studying them directly. It seems likely that the terminals may not be invaded by nerve impulses (73, 78). That nerve terminals may have different pharmacological properties from their axons or from the postsynaptic cells has been stressed by Riker and his colleagues (127, 163). More recently, it was clearly shown that the nerve terminals at lobster neuromuscular synapses have a specific pharmacology, different from that of the axon or the synaptic membrane (96, and unpublished). Phenylethylammonium diminishes the p.s.p.'s of the muscle fibers. It also blocks conductile activity in the motor nerves. However, when applied to the terminal region of the axons, in combination with other drugs (e.g. pictrotoxin) it causes reverberatory firing. An impulse arising in the axon at or near the ending sets up a train of antidromic activity. This effect occurs in both excitatory and inhibitory axons and also causes appropriate postsynaptic activity in the muscle fibers. Presumably the repetitive activity is caused by prolongation of the depolarization of the axon endings so that these regions act as "generators" somewhat like the receptive membrane of sensory neurons.

The agents that are believed to act on the nerve terminals in mammals (127, 163) are generally ineffective in lobster (96). Some ethonium ions increase the neurally evoked contractions of crayfish muscle (126), and it has been inferred, apparently by analogy with the data on mammalian preparations, that this action is also on presynaptic terminals. That conclusion is unjustified as long as electrophysiological data are absent. Onium ions are known to increase responsivenss of the electrically excitable membrane of crustacean muscle fibers (64, 200), and the mechanical response might increase correspondingly. Indeed, phenylethylamine has opposite effects on p.s.p.'s, decreasing their amplitude, and on nerve terminals, causing repetitive activity (96). It is regrettable that readily available electrophysiological techniques still tend to be neglected by pharmacologists.

The repetitive activity of the phenylethylamine-treated lobster axon is "inhibited" by γ-aminobutyric acid (GABA) and the inhibition is reversed by picrotoxin (96, and unpublished). These two agents respectively excite and block the inhibitory synapses of crayfish and lobster (30, 97), and of crayfish stretch receptors (124). However, they also affect the nonsynaptic membrane of crayfish muscle fibers (162) in essentially the same way, GABA increasing the permeability to Cl and picrotoxin decreasing it. Thus, it cannot be stated whether all the actions of the various drugs on presynaptic

terminals are on specialized secretory membrane or on some specialized component of transitional membrane between the conductile component and the secretory. The complexity in structure and function at junctions is illustrated in the diagram of Figure 1.

Still other complexities have been suggested by Eccles and his colleagues in a very extensive survey of the cat central nervous system (55 to 60, 62, 176, 177). The secretory output of the nerve terminals is believed to be modu-

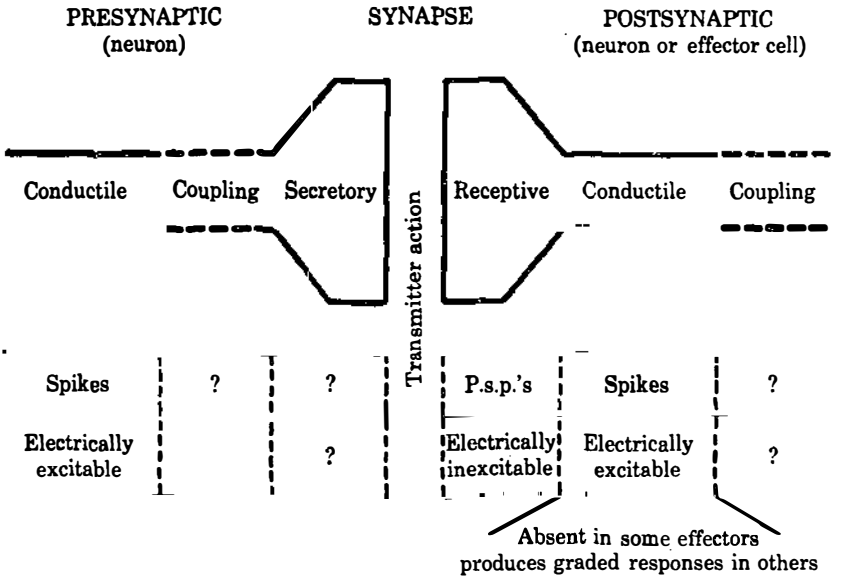

FIG. 1. Structural and functional components of synaptic transmissional activity in electrogenic excitable cells. The upper portion shows the different structural elements diagrammatically. The lower part describes the electrogenesis and type of membrane, as far as these are known. The receptor portion of the presynaptic neuron is omitted. It may be a synaptic or ephaptic junction or the receptive membrane of a sensory neuron. The depolarizing potential generated in the receptor or synaptic membrane, or induced in the postephaptic cell, initiates spikes which propagate in the electrically excitable conductile membrane. At or near the presynaptic terminals, which are believed to have secretory function, some unknown coupling mechanism translates the electrical signal into orders for secretory activity. After release of the transmitter and its diffusion across the synaptic cleft, transmitter action on the electrically inexcitable subsynaptic membrane is probably extracellular. The postsynaptic potentials (p.s.p.'s) generated by the synaptic membrane may be depolarizing (excitatory) or polarizing (inhibitory), or both types of activity may occur at different sites of the same cell. If the depolarizing p.s.p.'s are large enough, they can initiate conductile activity, providing the postsynaptic cells have an electrically excitable membrane component. Each of the different elements in the complex may be affected independently of the others and in more or less specific ways. Interference with any component thus results in changes in overall functioning. [From (85).]

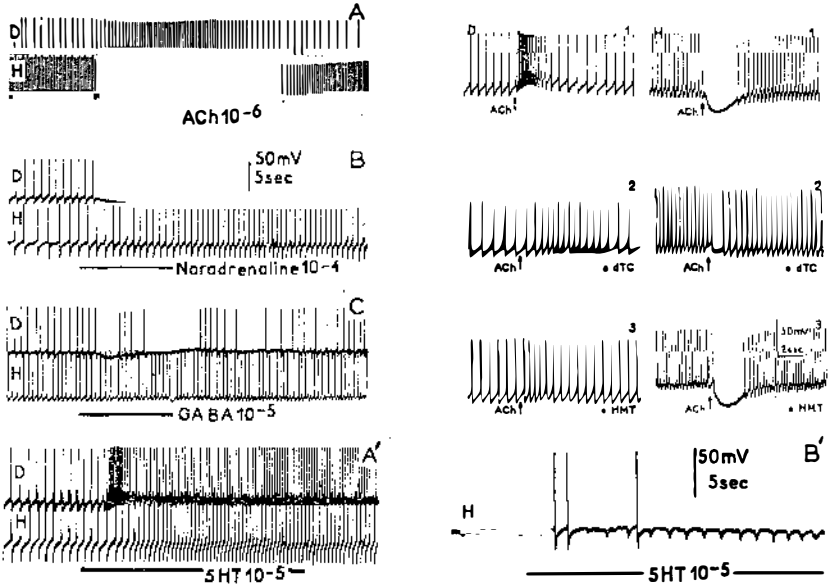

FIG. 2. Different pharmacological properties of two types of neurons of *Aplysia*. *Left:* Simultaneous recordings showing spontaneous activity of two cells characterized as D-neurons (*upper*) and H-neurons (*lower*), respectively. The bars under each set of traces indicate application of the specified agent. *Right* (*1–3*): Side-by-side presentation of recordings from D- and H-cells. In each case the arrow denotes an application of acetylcholine. The action of this agent on both cells was markedly diminished by a prior treatment with *d*-tubocurarine (*2*). However, treatment of the preparation with hexamethonium affected the response only of the D-cell (*3*). *B'*: The "diphasic" response of an H-cell to 5-hydroxytryptamine was an initial production of spikes, followed by a volley of i.p.s.p.'s. Explanation in text. [Combined from (75) and (191).]

lated by an innervation which impinges on the terminals, presumably at sites that are synaptic junctions. The nerve terminals are said to be depolarized as a consequence of this neural impingement, and the output of transmitter from the terminal is believed to be diminished thereby. This modulation has accordingly been termed "presynaptic inhibition." Pharmacological data have not as yet been published for the mammalian central nervous system (cf. 194; 175), but Schmidt (175) has cited a paper in press (61) and has discussed some of the results on the amphibian spinal cord in which "presynaptic" inhibition may also occur. A critical discussion of the data on presynaptic inhibition will be found in Bennett's review (21).

The studies on pharmacological reactions of *Aplysia* neurons (75, 191, 192), supplemented by data on *Helix* neurons (106 to 108), provide an excellent elementary summary (Fig. 2) of the principles involved in the action of drugs on synapses in terms of the concepts of the reviewer (81, 84, 85).

Some *Aplysia* neurons are depolarized by application of acetylcholine and have consequently been termed D-cells. Others, usually indistinguishable in other respects, are hyperpolarized and are termed H-cells. When spontaneous activity is present it is augmented in the D-cells and blocked or diminished in the H-cells. Thus, acetylcholine is an "excitant" of one type of cell and an "inhibitory" agent for another. In both actions, however, it is more exact to say that acetylcholine is an activator agent, of excitatory membrane in one case and of inhibitory in another. Likewise, norepinephrine is an activator of inhibitory membranes in D-cells and of excitatory in H-cells. This reciprocal relation, is, of course, also found in vertebrates.

The responses to the two activator agents are produced not only when they are applied to the synapses, which are located along the neurites as in neurons of many other invertebrates, but they are also obtained when the drugs are applied ionophoretically to the soma surface, which is free of synapses. Thus, the cell membrane can develop chemosensitive membrane independently of the presence or absence of innervation. Indeed, the D-cells do not have an inhibitory innervation, yet have chemosensitive (adrenoceptive) membrane with inhibitory functional capacity. It is likely that other excitable cells may also have patches of chemosensitive membrane, which is electrically inexcitable and which, being uninnervated, is apt to be undetected by electrophysiological tests alone (77). This type of membrane apparently occurs normally in mammalian nonmyelinated fibers (10, 11). It is also found in fetal muscle fibers and is produced along the extent of mammalian and frog muscle fibers which are denervated (12, 137) In denervated muscle fibers it is demonstrably electrically inexcitable (86).[4]

The chemosensitive membrane of *Aplysia* or *Helix* neurons does appear to be electrically inexcitable according to various criteria set forth by the reviewer (79, 83, 85, 92, 94). The inhibitory membrane is inverted to apparent depolarization by hyperpolarizing the cells (191, 192). Since it is dependent on Cl activation it becomes depolarizing when the external Cl is substituted with an impermeant anion (106), or when Cl or other permeant anions are injected into the cell (107). The change of anion in the medium or cell interior does not modify the resting potential significantly, and the depolarization of the "inhibitory" membrane now is excitatory in action (106, Fig. 5). Other examples of inhibitory membrane evoking excitatory activity are also known (85, 92). This is a consequence of the independent existence and properties of the two components.

Tauc & Gerschenfeld (192) are in error when they conclude that their finding of chemosensitive membrane runs counter to the distinction made between electrically excitable and electrically inexcitable membrane. The "chemically highly excitable somatic membrane" appears to be interspersed

[4] McLennan (136) reports this conclusion incorrectly. Only the chemosensitive patches, not the entire membrane of the denervated muscle fiber, become electrically inexcitable.

among electrically excitable components, exactly in the same way that synaptic electrically inexcitable membrane components are interspersed among electrically excitable conductile elements at the frog end plate (199), or among vertebrate neurons and electroplaques. Indeed, recent work by Tauc & Bruner (190) supports the view that the chemosensitive membrane of *Aplysia* neurons is electrically inexcitable. A region of membrane which is depolarized by acetylcholine becomes less responsive to a second application of the agent [desensitization (105)]. However, another region only about 50 μ away is not affected. The independence in reaction of nearby regions is a consequence of their electrical inexcitability and has been clearly demonstrated in the electrically inexcitable marine electroplaques (22). It is of considerable practical consequence, since heterosynaptic interaction must be hindered or made impossible thereby.

The *Aplysia* neurons respond differently to various other pharmacological agents. Thus, GABA depresses activity of the D-cells but has little or no effect on H-cells. On the other hand, 5-hydroxytryptamine causes depolarizing (excitatory) activity in both types of cells and in others as well. Kerkut & Walker (108) have tested the responses of 18 identified neurons in *Helix* to six agents which are among compounds that are frequently nominated as candidates for mediation of synaptic transmission or involved in the latter. Only eight of the cells responded to a single agent, four only to acetylcholine, two only to 5-hydroxytryptamine, one only to histamine, and one to DOPAmine. Seven neurons responded to two agents and three responded to as many agents but in different combinations. The likelihood that various neurons in any given region of the central nervous system respond differently to different agents has been confirmed in experiments with ionophoretic applications of drugs, as discussed below.

In record B′ (right) of Figure 2, is seen the complex result of such effects. The agent was "excitatory" not only for the H-cell, but also for some interneurons which activated the inhibitory synapses of the H-cell. The latter cell, which initially did not produce spikes because its inhibitory synapses were being bombarded (note small deflections at beginning of the trace), developed a few spikes during the direct excitatory effect of 5-hydroxytryptamine on it. However, the bombardment of the inhibitory synapses now became more intense indicating that the drug also excited the interneurons. The H-cell accordingly ceased discharging. Indirect effects of a drug must be expected to become still more prominent whenever the synaptic organization is still more complex. Burn & Rand (32), and Koelle (112, 113) have suggested that impulses which arrive at nerve terminals in autonomic systems release acetylcholine and that the latter than releases catecholamines. Thus, the action of acetylcholine would be an "indirect" one with respect to the effector cells themselves.

An additional specification of the pharmacological pattern of the *Aplysia* neurons is given by the different sensitivities of the D- and H-cells to the competitive inactivators of cholinoceptive membrane. The depolarizing

membrane of the D-cells is inactivated about equally well by *d*-tubocurarine (DTC) and by hexamethonium (HMT). However, the hyperpolarizing, inhibitory membrane is inactivated by DTC, but not by HMT in the concentrations used.

The concepts which are derived from data on such simple neuronal or neuroeffector systems can be used to account for the actions of drugs on the central nervous system (79, 81 to 83, 87, 91, 148). However, in order to achieve reasonably accurate explanations, consideration must be given not only to the specific characteristics of the individual neurons but also to their organization in structurally and functionally complex entities.

The studies with intracellular recordings from neurons of the vertebrate central nervous system cited above confirm the view that both e.p.s.p.'s and i.p.s.p.'s are generated by the cells. It is not unreasonable to suppose, further, that the excitatory and inhibitory synaptic membranes of neurons in the central nervous system also have different pharmacological specificities. To some degree these pharmacological reactions of the different membranes may be expected to resemble those of peripheral synaptic systems or the few varieties of neurons which have been studied in the spinal cord. This is not so generally accepted a view as are the first two propositions. Convulsive behavioral changes are induced in cats upon injection of drugs into the ventricles [see Feldberg (65) for older literature and Kumagai et al. (125) for the newer]. It has been suggested (65) that the pharmacology of cerebral neurons differs radically from that of peripheral systems. For example, injections of *d*-tubocurarine cause convulsions. However, under still simpler conditions, when electrocortical recordings were used (156), it was shown that electrographic signs of convulsions may be ascribed to the selective blockage of inhibitory synapses. In some regions of the neuraxis, or under some pharmacological conditions, *d*-tubocurarine appears to block inhibitory synapses preferentially and therefore can become a "convulsant" or "excitant" drug. A recent paper (138) reports that heparin protects the excitatory synapses of autonomic ganglia against blockage by *d*-tubocurarine as well as some other synapse inactivator agents, confirming an earlier finding on neuromuscular synapses (37). A similar effect of heparin has been suggested for the central nervous system (154, 156) to account for the differential effects of *d*-tubocurarine in the presence of heparin on cerebral and cerebellar superficial cortical responses. The similarity of action of this drug combination and of drugs which inactivate inhibitory synapses (strychnine, creatine) is striking (Fig. 3), and is strong evidence for the analysis presented above.

The potentials recorded during superficial cortical responses, augmenting, and recruiting responses are largely determined by the types and patterns of synaptic activity involved in the respective electrocortical recordings (3, 150, 158). The above cited work by Purpura and his colleagues, and forthcoming papers (159, 161) add further support to the view (79, 82, 88, 148, 152, 155, 156) that a dominant contribution to the electrical recordings from the cortical surface is that of the e.p.s.p.'s and i.p.s.p.'s of dendrites. Attempts to

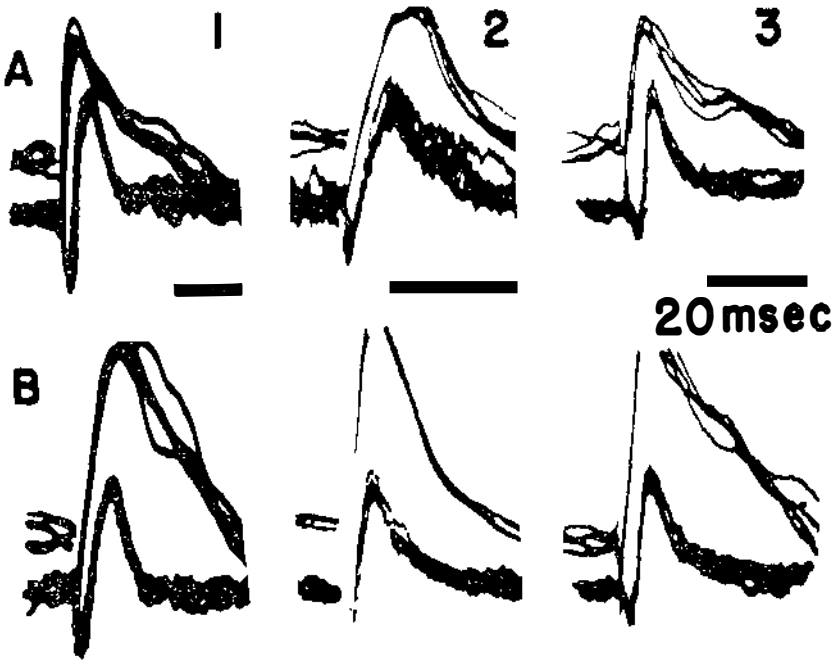

FIG. 3. The similar effects of diverse drugs, as shown in simultaneous recordings of superficial cortical responses (SCR's) from the cerebral cortex (the upper set of four superimposed traces) and the cerebellar cortex (the lower set). *A* shows the controls and *B* indicates the reaction after (*1*) the topical application of strychnine; (*2*) the injection of *d*-tubocurarine into the heavily heparinized animal; and (*3*) the topical application of creatine. In all three experiments the surface-negative cerebral SCR was augmented, while the cerebellar SCR was changed only minimally. Other data had led to the conclusion that both SCR's are p.s.p.'s of superficial dendritic synapses, that the surface negativity of the cerebral SCR was a composite of depolarizing e.p.s.p.'s which in general were larger than a simultaneously occurring hyperpolarizing and surface positive output of i.p.s.p.'s, and that the cerebellar SCR had little or no contribution of surface-positive i.p.s.p.'s. Effects of drugs on responses of the cerebellar cortex are shown and analyzed in Fig. 4. [Combined from (154) and (156).]

analyze recordings from the cortical depths encounter predictable difficulties (88). Li & Salmoiraghi (130) have provided fresh evidence on the difficulty of obtaining significant information from the so-called "d.c." or "steady" potentials of the brain.

There is still some controversy regarding the interpretation of pharmacological effects of topical application of drugs to the cortical surfaces. This mode of application has been used frequently because it may overcome the inaccessibility of neurons to many systemically administered drugs across the blood-brain barrier. However, it has also two other advantages. The applications may be limited to relatively restricted areas of the brain,

whereas systemic administration, if it is effective at all, usually involves many regions of the entire neuraxis. At the same time, however, topical applications also involve a large enough sample so that appropriate analytical treatment in terms of the neuronal populations can be meaningful. The results of topical applications can also be duplicated by systemic injections, when the electrical activity is studied at sites in which the blood-brain barrier has been breached (153).

The older data of the reviewer and his colleagues still present the most extensive series of studies on the pharmacology of cortical activity. A considerable number of drugs of different types of action have been tested on a variety of electrocortical activities under a number of different experimental conditions (151, 152, 154 to 156). The interpretations given then still appear to be valid and have the merit that a simple addition to the concepts described above permits formulation of an explanation which has a very high order of internal consistency.

The axodendritic synapses are postulated to have a different pattern of pharmacological reactivity from the axosomatic. This additional hypothesis derived from the fact that electrocortical activity could be modified by ω-amino and ω-guanidino compounds in various specific ways while conductile activity initiated by the neuron was essentially unchanged (154). Furthermore, the ω-amino and ω-guanidino acids and related compounds were assumed to be inactivators of the axodendritic synapses. Also, the short-chain compounds appeared to act on excitatory axodendritic synapses while the longer-chain compound acted on the inhibitory. Some degree of order was observed in data on strcuture-activity relations (154).

An interesting finding in this connection is the localized action of GABA in the Mauthner cell (50). The drug activates inhibitory synapses apparently only in the vicinity of the cell body and for a length of 200–300 μ lateral to the axon hillock. The extreme region of the lateral dendrite is insensitive to GABA. However, other inhibitory synapses probably occur along the Mauthner cell (72). All of them are blocked by strychnine.

The postulate of specific synaptic action of the ω-amino and ω-guanidino compounds has been challenged by a number of other investigators who employed electrocortical recordings, and most recently by Bindman et al. (26). These authors regard the effects of topical application as deleterious nonspecific actions. However, they employ a relatively complex type of electrographic data, responses evoked by stimulating sensory nerves subcutaneously. Furthermore, the experiments are limited in design, using rather few agents and only GABA as the amino acid drug. Much reliance is placed on depth recordings. The danger of drawing conclusions from complex electrocortical activities has been noted previously (152) and was again stressed recently (87, 88). The latter review also emphasized the need for sophisticated experimentation and for variety in drugs and responses (Fig. 4).

Early studies with ionophoretic application of amino acids to spinal neurons, summarized in Curtis & Watkins (46, 47), were regarded by Curtis

as decisive in rejecting the distinctions made between the effects of short- and long-chain ω-amino acids and related compounds (154). As ionophoretic studies have been extended to other neuronal varieties of the neuraxis and to frog and toad, the diversity of pharmacological patterns has come to be appreciated and the data have become far less clear cut. During the past year the following papers utilizing ionophoretic applications of drugs have appeared: (15, 17, 28, 31, 44, 45, 48, 118, 119, 120 to 123, 171, 182). A discussion, led by Salmoiraghi (170), in which others participated, is in press.

The earlier studies with the ionophoretic methods (see 46, 47) attempted to characterize the various effects of drugs with the use of intracellular recordings and with measurements of various parameters of the cell, such as its membrane conductance. This is a difficult task and, understandably, most recent investigations have concentrated on observing the effects of various applied drugs on the spontaneously active neurons. The cells are thus classified as nonresponding (usually the large majority) and those in which conductile activity is increased or decreased. Most of these observations offer little of theoretical significance and add almost nothing regarding the mode of action of drugs on neurons. Indeed, there seems to be the threat that such experiments may degenerate into a numbers game, with undue significance being attached to using a larger number of barrels in the electrodes, more drugs, and more cells observed.

Several theoretically significant studies may be singled out from the current literature. Curtis (42) has finally demonstrated that when strychnine is applied ionophoretically it does block inhibitory synapses. This is contrary to an earlier finding (47). Curtis now states (42) that the erroneous early report arose because i.p.s.p.'s were blocked by inadvertent leakage of strychnine from the electrodes. If the leakage artifact is eliminated, an effect of ionophoretically applied strychnine is readily observed. However, there is a disturbing question regarding the earlier work. The latter described "the absence of the effect of ionophoretically applied strychnine upon the i.p.s.p. . . . " (47, p. 441). In order to make so categorical a statement, the authors must have observed what they interpreted as i.p.s.p.'s after the application of strychnine. These earlier erroneous observations raise some questions on the accuracy of the intracellular recordings of i.p.s.p.'s from spinal neurons.

Krnjevic & Phillis who, like many others (see listing above), have demonstrated the presence of acetylcholine-sensitive cells in cerebral cortex (121) have then proceeded to characterize them more closely (122). These neurons seem to belong to the muscarinic variety of cholinergic systems, but they do not appear to differ markedly from other cortical neurons with respect to responses to amino acids and catechol- or indoleamines.

The structure-activity relations of cat spinal neurons as set forth by Curtis & Watkins (46) have been revised to some degree by later work (48). However, all the attempts thus far to make some order out of structure-activity relations, including those of the reviewer and his colleagues (154), have

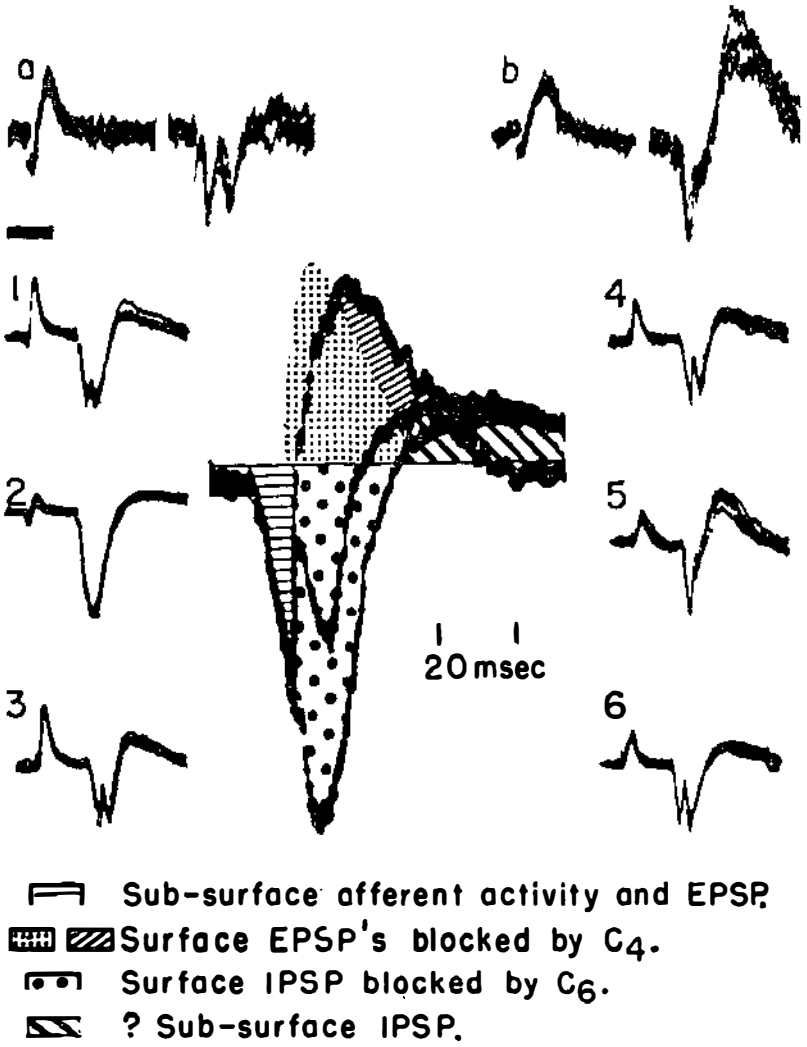

FIG. 4. An analysis of different pharmacological effects on different responses in one cortical region, the paramedian lobule of the cerebellum. In *a* and *b* are shown the effects of topically applied strychnine (0.1 percent solution). Effects of GABA are shown in *1*, *2*, and *3*; the effects of C_6 are shown in *4*, *5*, and *6*. Both of the latter agents were applied in 1 percent solutions.

Two cortical responses are shown in each record: the first is a predominantly surface-negative superficial cortical response (SCR) evoked by stimulating the paramedian lobule itself; the second is the cerebro-cerebellar response produced by stimulating the contralateral pericruciate cortex. This activity is composed of two surface-positive elevations, shown in *a*, *1*, and *4*. Strychnine (*b*) and C_6 (*5*) did not affect the SCR, but they eliminated the second positivity of the cerebro-cerebellar

not been conspicuously helpful. The studies with ionophoretic applications of various amino acids (44, 48) and other related compounds (45, 120, 123) have not proved any more successful. Furthermore, the effects of amines on cortical neurons (120 to 123) differ to some degree from the findings on geniculate neurons (45).

The extensive study of cortical neurons with the ionophoretic technique (120) disclosed that L-glutamic acid evoked or increased discharges in almost all neurons tested. Applications of GABA as promptly blocked the discharges reversibly. The rapidity of the action is stressed, in comparison with the slow onset of effects of other agents, which take 10 to 50 sec to achieve their maximum actions. However, D-homocysteic acid, which is a strong "excitatory agent," also requires about 20–30 sec for achieving its maximum effect on spinal interneurons (48). Krnjevic & Phillis (120) are inclined to regard the two rapidly acting agents as likely candidates for transmitters in the cortex.

However, the data are far from conclusive on this score, particularly since they involve external recordings using spike activity as the index of drug action. As has already been noted, the same agents may have effects on the nonsynaptic membrane components (85, 162). There is a suspicious "universality" about the actions of L-glutamate, which strongly suggests to the reviewer that the effect of this agent may be depolarization of the nonsynaptic membrane. Curtis & Watkins (48) also seem disinclined to regard L-glutamic acid as the normal excitatory transmitter. The data, likewise, do not permit a conclusion as to the mode of the action of GABA. Cessation of activity could occur by three different "inhibitory" modes, activation of inhibitory synapses, block of excitatory, or Cl activation of nonsynaptic membrane. Of interest also is the finding (120) that the long-chain ω-amino compounds have a rather nondescript effect. This one might expect if inhibitory synapses are blocked by the agents. The net effect would be a resultant in which various types of excitatory drives develop.

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response and brought out a large surface-negative potential. GABA eliminated the SCR (*2*) leaving behind a small diphasic component of action potentials. The cerebro-cerebellar response was augmented by the growth of its second positive component. The reversibility of these effects is shown in *3* and *6* about 15 min. after washing out the amino acids. The SCR of the paramedian lobule thus appears to be composed chiefly of surface-negative axodendritic e.p.s.p.'s as it is in other regions of the cerebellum (Fig. 3). An analysis of the cerebro-cerebellar response is shown in the inset (center) in which records *2*, *4*, and *5* are superimposed at an expanded scale. An early positivity and, perhaps, a late small negativity represent activity that is not affected by the amino acid drugs. A large component of the surface positivity ascribed to the axodendritic i.p.s.p.'s (large dots) is characteristic of the paramedian lobule and is disclosed by application of GABA. Blockage by C_6 reveals a smaller, normally obscured surface negativity (small dots) and an additional component of axodendritic e.p.s.p.'s (closely spaced diagonals) elicited by the stimulus in the absence of the i.p.s.p.'s. The time scale at the upper left is 20 msec for the experiment in *a* and *b*; it is 40 msec for the experiments in records *1* to *6*. [Modified from (151).]

If one takes into account the different experimental techniques, the effects which have been reported with many types of ionophoretically applied agents can be reconciled with the data obtained in topical applications (154). Ionophoresis probably results in application of relatively high levels of the agents in the region of the cell body and the basilar dendritic tree, with no effect on the dense population of superficial apical axodendritic synapses. Thus, the two types of measurement are probably exploring entirely different populations of synapses.

The synapses on the basilar dendrites, which are the superficial structures of the hippocampal cortex, do react to the ω-amino acids, although in a way somewhat different from that of the synapses of the apical dendrites of the neocortex (157). In the depths of the neocortex, the basilar dendrites are short relative to the apical tree. Thus, effects of drugs on the synapses of the basilar dendrite may be more readily translated into effects on the electrically excitable membrane than would be the case for the axodendritic influences upon the conductile membrane (79, 82, 83). This is merely a consequence of the "standing" character of the p.s.p.'s. This predicted difference has been observed in frog spinal neurons, which have specific inputs to axodendritic and axosomatic synapses (63).

Effects of the agents on the axosomatic synapses are not ruled out, of course, by the above interpretation of the data with ionophoretic applications. However, when systemic injections of the ω-amino compounds do affect electrical activity, at sites of blood-brain barrier impairment (153) the effects appear to be largely on axodendritic synapses. Data on relative sensitivities of different synaptic sites would be desirable.

Possible sources of error in inophoretic measurements have been discussed by Krnjevic et al. (118, 119) and by Spehlmann (182). A finding that the introduction of a small electrode into the auditory system increases the rate of "spontaneous" activity by about 20 percent (184) may also have implications for ionophoretic measurements. The multibarrel machines are formidable objects and might cause even more of this "spontaneous" presumably traumatic activity, or other effects.

The foregoing account reveals some of the difficulties that face attempts (e.g. 49, 139) at characterization of transmitters in the vertebrate nervous system. Some of the papers already listed, notably those of Curtis and his colleagues, discuss the problem in some detail (see 92). Kuffler and his colleagues have demonstrated the occurrence of GABA in the crustacean peripheral and central nervous structures (52, 116, 117). An enzyme system for synthesis of GABA has also been found (115). GABA is present in large amounts in inhibitory but not in excitatory axons of lobster. However, other substances (betaine, homarine, taurine) which are present ubiquitiously also have "blocking" effects. These findings, which are contrary, however, to data by Florey (68, 69), suggest that GABA might be a transmitter agent for some crustacean inhibitory synapses. That it cannot be such an agent in all was first pointed out by Florey & Hoyle (70), who found that GABA does not

increase the membrane conductance in crab muscle fibers. Nevertheless, GABA does diminish e.p.s.p.'s of the fibers. These findings have been confirmed (2). It has been suggested by that work that the inhibitory transmitter probably has two actions, one of activating inhibitory synapses, the other of antagonizing the effects of the excitatory transmitters.

Although the findings of Kuffler and his colleagues imply that GABA may be an inhibitory transmitter, i.e., one which activates inhibitory synapses, its action is nevertheless described as one of blocking activity. This usage is correct from the point of view of an operational description of the measurements, but in that case it also applies to betaine and other agents. Since the same or nearly similar overt effects can be produced by drugs of different modes of action, it is advisable to develop and adopt a more precise terminology.

The dynamics of ontogenetic modifications of structure and function still remain a rather neglected field of pharmacological research. Changes in structure, in electrophysiological properties, and of some pharmacological responses have been demonstrated by a few workers [see reviews by Purpura in 1961 (149) and in 1964 (160)]. An interesting new example is that of differential effects of different amines on chicks of various ages (109). Electrical and behavioral sleep is caused by administration of one group of these substances to chicks during their first month. The agents become relatively ineffective during the second month and thereafter cause arousal, both electrophysiological and behavioral. Another group of which amphetamine is a representative causes only arousal at all ages of the birds, while a third group is effective only after the second month and then also causes arousal reactions. These effects appear to be due to actions on systems of the brainstem which are differently organized at various times in the life of the animal (135). The similarity between ontogenetic changes and those effected by denervation and innervation of skeletal muscles is very striking (12, 137, 197, 198) and may have important bearing on the analysis of the dynamics of changes in properties (85).

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