1 May 1964, Volume 144, Number 3618 SCIENCE

Pharmacology of Individual Neurons

Microelectrophoresis provides closer observation of the interactions between nerve cells and drugs.

G. C. Salmoiraghi and F. E. Bloom

As awareness of the complexity of neuronal linkages within the brain becomes sharper, techniques of increasing complexity are devised for studying central-nervous-system activity. Among recent advances is the development of a method for restricting drug administration to the immediate external environment of a given nerve cell. Current studies are concerned with precise characterization of the response of individual neurons to various drugs with a view to gaining insight into the nature of those chemical substances (neurohumoral transmitters, or "transmitters," for short) that are released by nerve endings at their junctions with other cells (that is, at synapses). This article briefly considers the origin and nature of these investigations and indicates some of the problems which complicate the interpretation of drug action on cells of the central nervous system.

Neurophysiological Principles

Progress in neuropharmacology has depended upon the timely occurrence of conceptual and methodological advances in allied disciplines. In view of this intimate interdependence, a brief survey of basic neurophysiological principles is essential to establish a common orientation and terminology.

Microelectrode studies have shown that communication between neurons occurs by mechanisms similar to those responsible for transmission of impulses between nerve and muscle fibers (*1*, *2*). Stimulation of an excitatory nerve releases a transmitter substance which interacts with specific receptors in the subsynaptic patch of the cell membrane and causes it to become momentarily more permeable to certain ions. An inward flow of ions results in a reduction of the resting potential (that is, in depolarization). If depolarization reaches a critical level, an action potential is generated by the cell and propagated along its axon. Similarly, stimulation of an inhibitory nerve causes the release of a transmitter which brings about different ionic permeabilities, leading to transient hyperpolarization of the cell membrane. When the cell is in this state of hyperpolarization, depolarization by excitatory stimuli is less likely to occur.

Subsequent work (*3*) has shown that inhibition can also be effected without alteration of the membrane potential of the postsynaptic neuron. Thus, through changes in the level of polarization of the presynaptic terminals, these terminals become more or less capable of releasing their full quota of transmitter when the pathway is called upon to fire.

To recapitulate (see Fig. 1), a given neuron may be excited by the direct action of an excitatory transmitter (synaptic excitation), by potentiated release of transmitter from excitatory terminals (presynaptic excitation), or by inhibition of an inhibitory pathway (disinhibition). Inhibition of a neuron can result from direct action of the inhibitory transmitter (synaptic inhibition) or through dampening the release of transmitter from excitatory terminals (presynaptic inhibition). Finally, the possibility that electrical transmission may also be functionally important has recently been considered with renewed interest (*4*).

Pharmacology of Synaptic Transmission

The classical pharmacology of synaptic transmission arose from studies of peripheral organs in which nerve stimulation induced changes that could be monitored by means of relatively simple recording devices. Identification of the transmitters at these peripheral junctions as acetylcholine and norepinephrine and analysis of their mode of action made these junctions convenient model systems for pharmacological analysis. In turn, recognition of specific drug actions at sites with known transmitter provided pharmacological tools for identifying the transmitter at other junctional sites.

On the basis of studies of peripheral organs, rigorous criteria were developed for the identification of transmitters (*5*). These include proof of the existence of the suspected substance in presynaptic terminals and of its release by nerve stimulation. Additionally, the presence of appropriate anabolic and catabolic enzymes must be demonstrated, and it must be shown that administration of the substance mimics the action of the transmitter. Finally, it must

The authors are chief and research associate, respectively, in the Section on Neurophysiology, Clinical Neuropharmacology Research Center, National Institute of Mental Health, National Institutes of Health at Saint Elizabeths Hospital, Washington, D.C.

be shown that drugs which modify the effects of the transmitter modify the effects of the administered substance in identical fashion.

Fulfillment of these criteria, which requires convergence of several lines of biochemical and pharmacological evidence, is difficult in the peripheral nervous system and almost impossible at more central sites. Neurochemical analyses (*6*) thus far have mainly dealt with the irregular distribution in the brain of some biogenic amines known to have pharmacological action upon peripheral autonomic effectors. Recently, more direct evidence of their role as potential synaptic transmitters has been provided by the demonstration of their presence in brain extracts containing mainly axon terminals (*7*), and through histochemical analysis (*8*).

In pharmacological studies of the nervous system, drugs are usually administered intravenously, intra-arterially, or intrathecally. In some cases topical application or interstitial injection of potentially active material has also been attempted. For each of these various routes of administration, interpretation of the data obtained is quite involved. Drug molecules en route to possible central receptor sites encounter diffusional barriers (blood-brain, spinal fluid-brain, and interstitial) which may selectively interfere with their progression, and at any of several sites along their path they may encounter catabolic enzymes which can destroy them. But let us suppose that the drug reaches the central sites. There is a further difficulty: the exact time of arrival at reactive receptors cannot be directly determined, since the interval from time of administration to time of response is quite long (from several seconds to several minutes) in comparison to the time (on the order of milliseconds) required for transmission of information within the nervous system. As a result, it is difficult to establish that the observed effect was caused by action of the drug on the neural structure under immediate investigation, rather than indirectly through action of the drug upon peripheral structures or at other central sites, or even as an epiphenomenon of concomitant cardiovascular, respiratory, or metabolic alterations.

We find an additional set of complicating factors when we attempt to relate the accumulated pharmacological data (*9*) to the events of central transmission. Even for relatively uncomplicated synaptic sites—for instance, the neuromuscular junction—several potential mechanisms of drug action must be considered. For example, the drug may interfere with the synthesis, transport, or release of the transmitter, or it may prevent the released transmitter from affecting specific receptors on the subsynaptic membrane. Further, quite apart from its possible action at subsynaptic sites, the drug could affect the electrogenic components of either the nerve or the muscle-fiber membrane, or the contractile mechanism of the muscle, in such a way that conduction of impulses or contraction of the fiber, or both, (that is, functional transmission) would no longer be possible. Needless to say, one must bear in mind these same propositions in analyzing the action of drugs on more complex systems.

We shall now discuss some studies undertaken to provide evidence of direct neuronal effects of suspected synaptic transmitters. In these studies the drug is administered in the immediate vicinity of a given nerve cell, with only slight disruption of the integrity of surrounding elements.

Micromethods of Drug Administration

Several methods of drug administration have been proposed and tried. The micropipette used for recording electrical activity could be filled with the drug of choice, which would be administered at the proper time by pressure. The trauma would be only slightly greater than that produced by insertion of the recording pipette, and any reasonably nonviscous solution of drug or nonviscous extract or brain emulsion could be used. Practical attempts have shown that it is difficult to meet the strict requirements of single-unit recordings with this method of drug administration. Moreover, since the amount of drug released by the pressure pulse is related to the hydraulic resistence of the pipette, which is primarily determined by the size of the orifice of the tip, it follows that release through ultrafine micropipette electrodes of tip diameter less than 1 micron would require exceedingly high pressures (*10*). Use of pipettes with larger tips would permit spontaneous leakage of fluid and drug, which could result in gross errors in interpretation. In addition, any calcula-

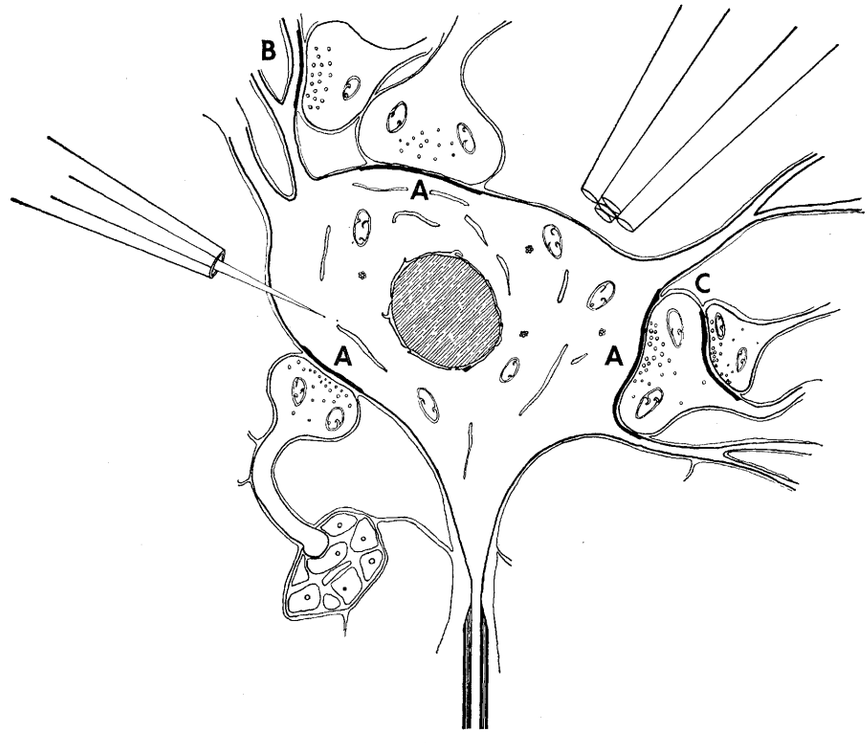

Fig. 1. Diagrammatic representation of a single neuronal unit, indicating three types of synaptic arrangements [axosomatic (*A*), axodendritic (*B*), and axoaxonic (*C*)] at which postsynaptic (*A* and *B*) and presynaptic (*C*) drug effects could occur. Nonsynaptic portions of the membrane are covered by glia. Subsynaptic patches are emphasized by thickening of the postsynaptic membrane. Tips of the two types of electrode assemblies (concentric and five-barreled) used in microelectrophoretic studies are drawn to approximate scale.

tion of the amount of drug released by a given pressure from a pipette of a given size would be liable to immediate invalidation when the electrode was inserted into the tissue, since partial occlusion of the pipette by tissue debris could reduce or prevent release of the drug by pressure.

A more practical micromethod of drug administration (*11*) is based on the well-known principle of migration of ions under the effects of an electrical field (electrophoresis). From a micropipette containing an aqueous solution of an ionizable drug, the biologically active ion (cation or anion, depending upon the chemical structure) can be ejected from the pipette, when desired, by current of the appropriate polarity. To a first approximation, when the electrical conductivity of the drug in the pipette is high as compared to that of the tissue fluid, the number of ions released is expressed by Faraday's law (*12*), according to which each nanoampere of current flow would eject approximately 1×10^{-14} gram equivalent of drug ion per second. More exact quantitation of the electrophoretic dose must await the determination of "efficiency" constants, which will express the ionic flux of each ionizable chemical achievable under actual experimental conditions (*13*). At present, dose can be merely estimated in terms of current flux, and devices (*14*) which will deliver relatively constant amounts of current are available to minimize the effect of variations in the electrical resistance of the micropipette in the course of the experiment. Spontaneous diffusion of drug can be checked by the application of a retaining current (of polarity opposite to that of the ejecting current), which holds the active ion within the micropipette. Needless to say, careful controls are necessary to rule out the effects of *p*H, electrical current, and potential actions of the complementary drug ion. Microelectrophoresis has partially overcome the limitations of classical neuropharmacological techniques, but additional modifications have been required in its practical application.

The microelectrophoretic technique was first applied during in vitro studies on chemical transmission at the neuromuscular junction (*15*), where it was possible to position several micropipettes independently under visual control. Ultrafine recording microelectrodes could be inserted into muscle fibers close to an end plate (the neuromuscular synapse) or some distance away along the muscle fiber. Micropipettes containing drugs were used to eject ions, directly at the end plate or at other points along the nerve and muscle fibers. It was possible, therefore, under these experimental conditions, to compare the effects of microelectrophoretically administered acetylcholine with the effects of motor-nerve stimulation on the precise conductance (that is, ion-permeability) changes occurring in the junctional area. In addition, the effects of known acetylcholine antagonists, cholinesterase inhibitors, and neuromuscular blocking agents could be compared on the same parameters of transmission. These experiments were instrumental in establishing the identity of the transmitter at the neuromuscular junction as acetylcholine. Recently this approach has been extended to the study of another set of peripheral cholinergic synapses, the sympathetic ganglia of the frog (*16*).

Characterization of a Transmitter in an Elementary Nervous System

The usefulness of the microelectrophoretic technique has also been demonstrated by recent in vitro studies of ganglion cells of the marine mollusk *Aplysia* (*17*). Neurons of this elementary nervous system are relatively large; their size and the anatomical arrangement of their synaptic contacts permit independent positioning of two or more micropipette electrodes within and outside the cell, similar to the positioning of electrodes in the studies on transmission at the neuromuscular junction. The conductance changes resulting from release of the actual transmitter could thus be compared with the changes induced by drugs injected into the organ bath or administered microelectrophoretically on synaptic or nonsynaptic portions of the membrane.

These experiments showed that the effects of acetylcholine duplicated the effects of the natural transmitter for many of the *Aplysia* ganglion cells. The responses to acetylcholine and the functional transmission were similarly modified by injection of synergists and antagonists of acetylcholine into the organ bath. In the cholinoceptive cells, both acetylcholine and the transmitter were capable of producing either of two effects: depolarization of some cells (called D cells) and hyperpolarization of others (H cells). In this system of cells, therefore, acetylcholine has a dual transmitter role, excitation and inhibition. Since the transmitter is the same in the two cases, it is clear that the direction of the cell response must depend upon the characteristics of the synaptic receptor membrane. This finding is not without biological precedent [for example, depolarization of skeletal and hyperpolarization of cardiac muscle fibers by acetylcholine (*18*)].

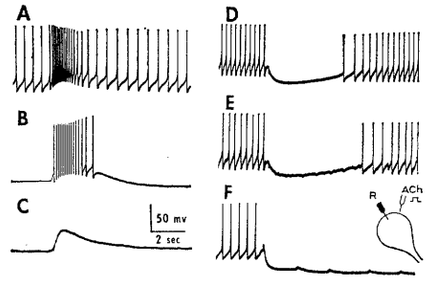

Fig. 2. Effects of brief (200-msec) electrophoretic administration of acetylcholine on somatic membrane of a D cell (*A*, *B*, and *C*) and of an H cell (*D*, *E*, and *F*) at increasing levels of membrane polarization (*A* through *C*; *D* through *F*). The cells are *Aplysia* ganglion cells. (Inset at right) Position of intracellular recording electrode (*R*) and pipette for extracellular administration of acetylcholine (ACh). [Modified from Tauc and Gerschenfeld (*17*)]

Moreover, when acetylcholine was administered electrophoretically to the nonsynaptic portions of the somatic membranes of D or H cells, it still produced the same dual effects (Fig. 2). This interesting observation implies, first, that the membranes of these neurons have profoundly different properties of chemical responsiveness. In addition, the apparent existence of chemical sensitivity at sites on the membrane not immediately subjacent to synaptic endings suggests that any functional specialization possessed by the subsynaptic patches is shared to some extent by the remainder of the somatic membrane.

In vivo Study of the Mammalian Central Nervous System

Unfortunately, the technical and conceptual advances stemming from the in vitro studies cannot be immediately applied to the analysis of central-neuron phenomena, which must be studied in vivo. One point of obvious difference is that exploration of the brain with microelectrodes is essentially a blind procedure. Although a general area of the nervous tissue can be preselected by

Table 1. Responses of neurons in various sites to chemical substances administered by means of microelectrophoresis. F, Facilitation; D, depression; N, no response.

Neuron	Drug							References
	Acetylcholine	Norepinephrine	Dopamine	5-HT	Amino acids: Glutamate	Amino acids: GABA	Others	
Aplysia	F D							(*17*)
Snail	F D				F D	F D		(*43*)
Frog sympathetic ganglia	F							(*16*)
Spinal cord:								
Motoneurons	N		N		F	D		(*21, 22*)
Renshaw cells	F		N		F	D		(*21, 22, 24, 25, 44, 45*)
Interneurons	N		F N		F	D	Histamine	(*21, 22, 28, 45*)
Medulla	F N D	F N D						(*46–48*)
Pons	F N D	F N D						(*47, 48*)
Inferior colliculi	F N	N		N	F	D		(*47*)
Hypothalamus	F N D	F N D		F N D				(*49*)
Thalamus:								
Ventrobasal complex	F		N	N D	F	D		(*50*)
Lateral geniculate	F	D	D	D	F	D		(*29, 30*)
Caudate	F N D	F N D	F N D					(*40*)
Cortex:								
Auditory, somatosensory, sensory-motor	F N	D	D	F* D	F	D	Epinephrine	(*33*)
Visual	F N	D	D	F* D	F	D		(*32, 33*)
Cerebellum	F				F†	D		(*33, 51*)
Olfactory bulb	F N D	F N D		N D	F D			(*37–39*)

* Large dose. † Homocysteic acid.

means of topological landmarks, estimation of the position of the electrode tip relative to a given neuron must rest solely on observations of the nature of the recorded potentials (*19*). Detection of neural units depends upon either spontaneous activity or activity induced by stimulation of a specific neural pathway. The ease with which cells can be encountered with the electrode tip is related in part to the size and type of the microelectrode used and to the surgical or chemical means by which the animal has been made insensitive to the manipulations required for exposure and immobilization. It is clear that, in this situation, independently positioned micropipettes would be relatively useless for pharmacological investigations of the types just discussed, since the chance that all their tips could be concurrently maneuvered onto the desired portions of the same neuron within the depths of the brain would be infinitesimally small.

One possible solution for this problem was offered by the development of a concentric assembly of two micropipettes (*20*) arranged so that the tip of the smaller one protrudes some 50 to 100 microns from the orifice of the larger, drug-containing, pipette. This concentric system makes possible simultaneous extracellular electrophoretic administration of drugs and intracellular observation of electrical events (Fig. 1). In the only extensive trial of this method to date, D. R. Curtis and his co-workers (*21, 22*) analyzed the conductance changes, in spinal motoneurons, produced by microelectrophoresis of many substances, including certain naturally occurring amino acids which have potent excitatory and inhibitory effects upon crustacean muscles (*23*). The relatively smaller size of other central neurons and the difficulty of preventing interference from hemodynamic and respiratory movements may explain why the concentric micropipette approach has not had wider application.

Curtis and R. M. Eccles (*24, 25*) offered a more practical solution, but one which restricts the observation of electrical events to those detectable with extracellular microelectrodes. By fusing together several glass tubes of small diameter and drawing them out simultaneously, they prepared a multibarreled glass micropipette (Fig. 3). The orifices of the various components of this assembly were side by side at the tip, and the outer, drug-containing components were equidistant from the central, recording pipette. The most commonly used multibarreled cluster (the five-barreled glass micropipette electrode) can be made with an overall tip size of 3 to 8 microns. This is sufficiently small to permit extracellular recording of action potentials of individual neurons while permitting controlled electrophoresis of drug from the outer four barrels.

Microelectrophoresis in the Study of Central Neurons

Since the development of the multibarreled micropipette electrode, many chemical substances have been tested on a wide variety of nerve cells. The data of Table 1 show the correlation between the neuroanatomical sites in which cells have exhibited local sensitivity to chemicals and the substances most frequently administered electrophoretically in these tests, and it indicates the types of responses which have been observed. We have selected five of the studies of Table 1 for consideration in greater detail, in order to illustrate the possible means of gaining insight into the nature of chemical responses of central neurons.

Extensive microelectrode investigations by J. C. Eccles and his co-workers clearly demonstrated the functional importance of spinal interneurons in the control of motoneuron activity (*1*). One type of interneuron, the Renshaw cell (*26*), is monosynaptically excited by axon collaterals of the motoneuron, which is in turn monosynaptically inhibited by the Renshaw cell. The activity of cells of both types can also be regulated through other synaptic pathways. From the effects of parenterally administered acetylcholine and related drugs, it appeared likely that acetylcholine was involved in the excitation of Renshaw cells (*27*). This body of information permitted Curtis and R. M. Eccles (*24, 25*), in the original application of the multibarreled micropipette method, to utilize a well-studied system providing ready access to neural pathways, electrical stimulation of which produced predictable effects upon the cells these workers wished to study.

Through local electrophoretic administration of acetylcholine and drugs which in peripheral systems mimic, potentiate, or block the action of acetylcholine, it became apparent that acetylcholine was acting in the spinal cord at the synapse by which Renshaw cells are excited by motoneurons. Both electrophoretic administration of acetylcholine and motor-nerve stimulation produced excitation of Renshaw cells. In addition, Prostigmine, which inhibits the action of cholinesterase, prolonged the effects of electrophoretically administered acetylcholine and potentiated the effectiveness of the synaptic stimulation. Finally, *d*-tubocurarine and dihydro-β-erythroidine, which blocked the response to acetylcholine, grossly

reduced the excitation produced through the synaptic pathway (Fig. 4).

Subsequent attempts by Curtis *et al.* (*22*) to demonstrate sensitivity of interneurons and motoneurons to this and other biogenic amines were unfruitful. However, electrophoretic administration of Dopamine has recently been reported to cause excitation of certain spinal interneurons associated specifically with the reticulospinal pathway (*28*).

Another detailed set of experiments concerns the relay station of the visual system, called the lateral geniculate nucleus. At this point in the pathway, neurons can be made to discharge by stimulation of optic nerve fibers, or through retrograde invasion by stimulation of their efferent terminations. Although these cells respond to microelectrophoretic administration of many naturally occurring substances, including acetylcholine and glutamate (*29*), the responses to serotonin (5-HT) are particularly noteworthy. Administration of the latter compound decreases the rate of spontaneous discharge and, in addition, prevents excitation of the cell by visual nerve stimuli while not suppressing responses to retrograde invasion (Fig. 5). Curtis and Davis (*30*) have interpreted these findings as implying that 5-HT either blocks the access of the excitatory transmitter to subsynaptic receptors of lateral geniculate neurons or prevents release of the excitatory transmitter from optic nerve terminals. The latter possibility is compatible with a function for 5-HT in this system as a presynaptic inhibitory transmitter (*31*).

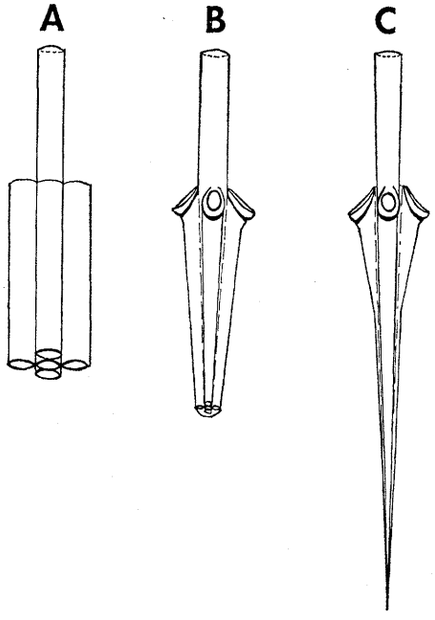

Fig. 3. Steps in the preparation of five-barreled glass micropipette electrodes. Four glass tubes are fused around a central tube (*A*) and partially drawn out by hand (*B*). In the final step (*C*) the electrode is further drawn out to tip size of less than 0.5-micron diameter by commercially available microelectrode pullers. The tips are enlarged to 3- to 8-micron diameter prior to use.

At the cortical terminations of the visual pathway, Spehlmann (*32*) has demonstrated that microelectrophoretically administered acetylcholine is capable of increasing the spontaneous discharge rate of many neurons. During the administration of acetylcholine the cells were discharged more easily by visual or electrical stimulation. In addition, many of the responses to acetylcholine could be potentiated by Prostigmine, a cholinesterase inhibitor. Krnjević and Phillis (*33*) investigated the chemical responsiveness of neurons in sensory and motor areas elsewhere in the cortex. While the majority of the neurons tested exhibited sensitivity to amino acids such as glutamate or gamma-aminobutyrate, acetylcholine-sensitive cells were more prominent among neurons identifiable as Betz cells (cortical motoneurons) on the basis of their electrical response to stimulation of the pyramidal tract. In view of these microelectrophoretic studies of the cortex, and of earlier reports that acetylcholine is released from functionally active cerebral tissue (*34*) and that cholinesterase can be demonstrated in this area by histochemical methods (*35*), it seems likely that acetylcholine has a functional cortical action, although the exact synaptic site remains to be demonstrated.

A different neurotransmitter is implicated in the last set of data to be considered. In the olfactory bulb the activity of many cells is suppressed by stimulation of the lateral olfactory tract, which consists mainly of axons of one type of olfactory cell, the mitral cell. Mediation of this inhibitory pathway is believed to be through activation of mitral-cell axon-collaterals (*36*). These cells respond to microelectrophoretic administration of norepinephrine, acetylcholine, or 5-HT, predominantly by exhibiting slower rates of discharge (*37*). When the effects of norepinephrine on mitral cells are blocked by electrophoretically administered norepinephrine antagonists, the effectiveness of the lateral-olfactory-tract inhibitory pathway is concomitantly reduced (Fig. 6) (*38*). On the basis of this and other evidence, we feel it likely that norepinephrine has synaptic transmitter functions in this neuronal system (*39*).

Precautions in Interpreting Microelectrophoretic Data

Clearly, an abundance of data on the chemical responsiveness of central neurons is now available. It is apparent, therefore, that the technique of microelectrophoresis is of unquestionable assistance in studying the pharmacological responsiveness of nerve cells, chiefly because it is a means of bypassing some of the major diffusional barriers and of greatly reducing the number of potential sites of drug action. However, the information which is provided must be interpreted judiciously.

When a drug has no demonstrable effect on a particular type of neuron, one should probably not take this to mean that it definitely has no effect. We say this for several reasons. First, since exploration of the brain with microelectrodes cannot be carried out under conditions of

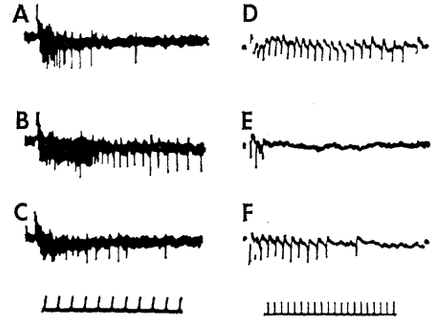

Fig. 4. Effects of electrophoretic administration of Prostigmine and of dihydro-β-erythroidine on synaptic excitation of Renshaw cells by motor-nerve stimulation. (Records at left) Response to Prostigmine: *A*, control; *B*, 2 minutes after ejection; *C*, 17 minutes after ejection. (Records at right) Response to dihydro-β-erythroidine: *D*, control; *E*, 70 seconds after injection; *F*, 28 minutes after injection. Time markers for records *A–C*, 10 milliseconds; for records *D–F*, 1 millisecond. [Modified from Curtis and Eccles (*25*)]

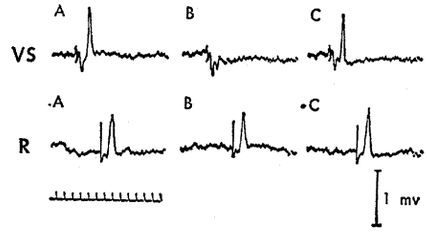

Fig. 5. Response of a single lateral geniculate neuron to synaptic excitation through the visual pathway (*VS*), or to retrograde invasion (*R*), (*A*) before, (*B*) during, (*C*) after electrophoretic administration of 5-HT. Time markers, 1 millisecond. [Modified from Curtis and Davis (*30*)]

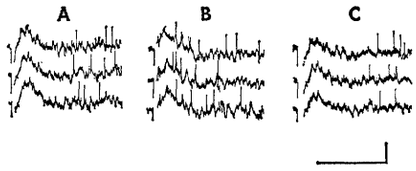

Fig. 6. Response of a single mitral cell in the olfactory bulb to synaptic inhibition by stimulation of the lateral olfactory tract, (*A*) before, (*B*) during, and (*C*) after electrophoretic administration of a norepinephrine antagonist (dibenamine). Calibrations: 200 microvolts and 150 milliseconds. [Modified from Bloom *et al.* (*38*)]

visual control, potentially reactive cells may be completely missed, because of their size and rates of discharge, in exploring the brain of a given animal. Moreover, even if accurate determination of the distance of the electrode tip from the recorded neuron were possible, uncertainties relating to the quantity of drug ejected and to the decremental effects of interposed catabolic enzymes and "synaptic barriers" (*24*, *25*) would make it impossible to obtain precise quantitive data on the effectiveness of the drug. Additionally, it is possible that anesthetic agents commonly used in experiments of this type may modify or block otherwise observable response (*40*). For these reasons we give no quantitative estimates of responsiveness, although such estimates have been published (see *21*, *29*, *30*, *33*).

As other reviewers have observed (*41*), mere demonstration of the effect of an electrophoretically administered substance on either spontaneous or induced activity does not immediately indicate which of the many possible cellular sites of drug action is involved. Presynaptic and postsynaptic sites of drug action probably could be distinguished by simultaneous intracellular recording and extracellular drug administration, if only the method were more practicable. With current microelectrophoretic techniques there is often a delay of several seconds between the occurrence and the recording of an effect, a delay which makes it difficult to distinguish between synaptic or nonsynaptic sites of action on the recorded nerve cells and similar sites on a functionally related neighbor. Although this latency may be partly biological, it is probably attributable in part to the retaining current and to accumulation of tissue debris around the tip of the microelectrode.

The use of pharmacological agents with synergistic and antagonistic effects has proved helpful in characterizing suspected transmitters. However, the specific effects of the modifying drug upon the transmitter substance at a given central site cannot be generalized to other central sites without further proof, since it is well known that certain peripheral synapses operated by the same autonomic transmitter show a wide range of specificities and responsiveness (*42*). To date, the best characterization of transmitters at central sites has been obtained in studies of the Renshaw cell, of the lateral geniculate nucleus, of neurons in the cortex, and of the olfactory bulb, because in each of these cases the state of physiological understanding of local synaptic relations was sufficiently far advanced to allow comparative pharmacological studies.

No technique now available is, per se, the "ideal" tool for pharmacological investigation of neurons. It would seem that certain yet untried combinations of techniques may be needed for a deeper understanding of basic phenomena. One potentially fruitful approach might be the use of microelectrophoresis followed by histochemical or cytochemical analysis to learn more about the biochemical substrate responsible for the cell's pharmacological response. Obviously, in taking this next step, no matter how logical it appears, we will again be faced with the difficulties arising from a need for new skills and techniques.

Summary

We are in a transitional period, in which older techniques for studying the pharmacological responsiveness of nerve cells are giving way to newer methods as a result of a deepening awareness of the complexities of central-neuron organization. A recent approach, that of electrophoretic administration of chemical substances in the immediate proximity of individual nerve cells, has proved useful, in spite of the difficulty in interpreting the findings that results from our incomplete knowledge of synaptic arrangements in the many regions of the brain where this method was tried, and in spite of the method's inherent technical limitations. Advances may be expected in the near future, at a quickening pace, as soon as other model systems of central synaptic arrangements become available.

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52. We thank Drs. D. R. Curtis and L. Tauc, and the editors of the *British Journal of Pharmacology and Chemotherapeutics*, the *Journal of Neurophysiology*, the *London Journal of Physiology*, and *Life Sciences* for permission to reproduce illustrative material.

Prehistory of the West Indies

The Indians Columbus encountered when he discovered the New World were moving up from South America.

Irving Rouse

The islands of the West Indies are of interest to prehistorians because they lie like a series of stepping stones between the northeastern part of South America and the peninsulas of Florida and Yucatan, projecting from North and Middle America, respectively (Fig. 1). From which of these three mainland regions did the Indians reach the islands? When did various groups of Indians first arrive? Did they continue through the islands into other mainland regions? And did certain customs and beliefs spread to or from the islands without an accompanying displacement of the population? Research on the prehistory of the West Indies is designed to answer these questions (*1–3*).

The islands have also attracted attention because they were the scene of the first significant contacts between the Old and New Worlds. The earlier Norse encounters with the Eskimo had contributed nothing to the development of western civilization, but when Columbus discovered the New World in the West Indies, he set in motion a chain of events which led to the adoption by Europeans of a number of new crops, such as maize and tobacco, and new artifacts, such as hammocks and canoes, which we now consider our own. Even the names for these crops and artifacts are taken from the West Indian languages. It is of some interest, therefore, to determine how they reached the islands.

The author is professor of anthropology at Yale University, New Haven, Conn.

Natural and Cultural Setting

The Lesser and the Greater Antilles form the backbone of the West Indies. The Lesser Antilles consist mainly of small, volcanic islands, which curve to the north and west from the mouth of the Orinoco River in eastern Venezuela (Fig. 1). The Greater Antilles, composed of much larger, mainly sedimentary islands, extend westward from the northern end of the Lesser Antilles toward Florida and Yucatan. From east to west, the principal islands of the Greater Antilles are Puerto Rico, Hispaniola (which is now divided between the Dominican Republic and Haiti), Jamaica, and Cuba.

Lesser island groups include the Turks and Caicos Islands and the Bahamas, scene of Columbus's first landfall;