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New concepts of molecular communication among neurons

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Abstract: Recently a number of complex electrophysiological responses to neurotransmitters have been observed that cannot be described as simple excitation or inhibition. These responses are often characterized as modulatory, although there is no consensus on what defines modulation. Morphological studies reveal certain neurotransmitters stored in what might be release sites without synaptic contact. There is no direct evidence for nonsynaptic release from CNS sites, although such release does occur in the periphery and in invertebrates. Nonsynaptic release might provide a basis for diffuse one-cell-to-many communication, but it might also simply be a means of sending the transmitter to a broader area of a single neuron than occurs in typical synapses. Several kinds of macromolecules have been found to be transported in a retrograde direction – and in some cases transsynaptically. There have been suggestions that some neurons may release more than one type of transmitter. Particularly intriguing is the possibility of release of substances that modulate actions of a primary transmitter. Taken together this range of evidence suggests that neurons may use a variety of forms of molecular communication in addition to traditionally described synaptic transmission.

Several authors have suggested modes of communication distinct from classical synaptic transmission and have classified released substances using terms such as neurohumor, neurohormone, neuroregulator, and modulator. These suggestions have the heuristic value of drawing together diverse kinds of data, but it remains to be established that the pieces fit together in that fashion – for example, that complex electrophysiological effects are associated with substances released nonsynaptically. In order to reduce confusion, a flexible, generic approach to nomenclature for substances released from neurons and for hypothetical modes of communication is recommended. Some behavioral implications of nonconventional transmission are considered.

Keywords: amines; Dale's Principle; modulators; neurohormones; neurohumors; neurotransmission; neuropeptides; synapses

The notion of synaptic chemical communication is central to our conceptualization of neuronal function. As early reticular theories of the nervous system were displaced by the concept of the neuron as a discrete functional unit, it became evident that there must exist specialized points of contact for passing information from one cell to the next (Eccles 1964 and ref. therein). The term "synapse" was coined by Sherrington (1906), who recognized, almost presciently, that crucial features of the operation of neuronal circuits could be explained by the character of synaptic transmission. For example, he argued that excitatory and inhibitory inputs would be algebraically summated by a postsynaptic neuron, and that a subthreshold stimulus repeated rapidly would be summated, causing firing.

The concept of synaptic transmission helped bring together the classical tenets of the neuron doctrine that emerged in the first half of this century. These tenets suggested that neurons, dynamically polarized into distinct receiving and transmitting zones, summate their excitatory and inhibitory synaptic inputs and transmit information in all-or-none impulses along axons, whose terminals release a single kind of neurotransmitter. These principles were shown to have broad applicability and provided a conceptual framework for understanding diverse aspects of nervous system function. However, the early period of apparent clarity and simplicity evoked by successful theories in biology is often followed by a time of increasing complexity as exceptions and inadequacies are discovered. Thus, for example, twenty years ago Bullock (1959) pointed out the need for revision of textbook statements of neuron doctrine, particularly in regard to the integration of postsynaptic responses and the generation of action potentials; it was becoming apparent that dendritic potentials interacted in a way more complex than simple summation of excitatory and inhibitory influences to elicit propagated impulses. His views have been well borne out by developments in the last two decades. For example, some interneurons in various regions of the brain have been found to exhibit such unexpected features as synapses between dendrites, reciprocal synapses, and bidirectional transmission of graded potentials rather than impulses (Rakic 1975).

Over the past several years, certain neurotransmitters have been reported to elicit, in various neuronal systems, complex electrophysiological responses that cannot be simply described as either excitatory or inhibitory. The word "modulation" has often been used to describe these complex responses, as well as a number of other kinds of alterations of neuronal activity. Several putative neurotransmitters, particularly norepinephrine, serotonin, and certain neuropeptides, have been suggested to be neuromodulators or neuroregulators, but these terms have generally been used ambiguously and often in conflicting ways by different authors, and it is not clear how modulation is to be distinguished from other neural actions.

Recently, certain morphological evidence has been interpreted as suggesting that some neurotransmitters may be released from some sites nonsynaptically, for diffusion to targets more distant than those found in conventional synaptic transmission. Barker (1977; 1978) and Chan-Palay (1977) have combined the concept of nonsynaptic transmission with evidence for complex electrophysiological responses in suggestions that there may occur specific modes of molecular communication among neurons distinct from classical synaptic transmission. These suggestions have raised a certain amount of controversy. There is not yet direct evidence for nonsynaptic release from central neurons, and even with such evidence many questions will remain about the targets of such release and the functions served. Nevertheless, a range of evidence from various neuronal systems suggests that neurons communicate and process information in ways that go beyond traditional concepts.

The objectives of this article are:

1. To critically examine evidence for nonclassical modes of molecular communication among neurons. Are suggestions for distinct modes premature,

or do they have heuristic value?

2. To see what common threads can be found among the various ways in which the concept of modulation has been applied to neuronal function.

3. To examine implications of this research for the ways in which we formulate principles of neuronal operation.

I will not attempt in this article to review the great wealth of information now available about synaptic organization and function; broad summaries of that sort have already been made (e.g., Shepherd 1974). Rather, I will try to draw suggestions together that have been made about nonconventional transmission and other kinds of evidence bearing on concepts of neuronal communication. Hopefully this will provide a better context for evaluating these suggestions and discussing their implications for neuronal operation in the Commentary.

Can neurotransmitters be released nonsynaptically?

The possibility of nonsynaptic release has been suggested for several amine neurotransmitters, particularly serotonin and norepinephrine. These two putative transmitters are contained primarily in pathways arising from small brainstem nuclei and branching diffusely to innervate much of the brain (see Moore & Bloom 1978). In both noradrenergic and serotonergic pathways the transmitter is not just contained in nerve terminals but is also concentrated in varicosities closely spaced (1–3μ) along tiny unmyelinated axons. Electron microscopy (EM) has revealed these varicosities to contain aggregates of small, round, agranular "synaptic" vessels, a few large granular vesicles, and mitochondria (see ref. in Descarries et al. 1977; and Descarries et al. 1975). Thus, varicosities of aminergic axons contain apparatus usually associated with the storage and release of transmitters.

Several ultrastructure studies, using different techniques for tagging amine-containing cellular processes, have indicated that some axonal varicosities make synaptic contact, but the majority do not appear to do so. Low incidence of synaptic contact by varicosities has been reported for catecholamines (Ajika & Hökfelt, 1973) and indolamines (Calas et al. 1974) in the median eminence, serotonin in cerebral ventricles (Richards et al. 1973; Chan-Palay 1976), presumptive dopamine in the neostriatum (Tennyson et al. 1974 and refs. therein), and serotonin (Descarries et al. 1975) and norepinephrine (Descarries et al. 1977) in the neocortex.

Detailed studies of aminergic axonal processes in the neocortex used the technique of EM-level radioautography. In these experiments ^{3}H-serotonin (Descarries et al. 1975) or ^{3}H-norepinephrine (Descarries et al. 1977) was topically applied *in vivo* to the neocortex to allow cellular processes to become labelled by active uptake. The relatively high concentrations of ^{3}H-amines used in these procedures call for careful controls to demonstrate that label has not accumulated in nonaminergic cells through nonspecific uptake. This was accomplished, for example, in the norepinephrine study by demonstrating that labelling of axonal varicosities was prevented by pretreatment of the animals with desmethylimpramine, to selectively block high-affinity uptake of norepinephrine, or with 6-hydroxydopamine, to selectively destory noradrenergic neurons. Similar controls were performed in experiments with ^{3}H-serotonin.

In these neocortex experiments ^{3}H-norepinephrine and ^{3}H-serotonin appeared to accumulate specifically in axonal processes, particularly varicosities. Label appeared most concentrated over intracellular organelles, especially the small agranular vesicles. Extensive topometric ultrastructural analysis was performed on serial sections, making possible two or even three sequential sections through a large number of varicosities.

A striking finding of these studies was that less than 5% of either ^{3}H-serotonin- or ^{3}H-norepinephrine-labelled varicosities evinced synaptic contact. In contrast, synaptic junctions *were* seen in some 50% of unlabelled boutons in the immediate vicinity. It is not possible to tell exactly what percentage of either labelled or unlabelled boutons actually made synapses, since many junctions would be missed by the sections. The junctions observed with aminergic varicosities were not of unusual size, and no differences of internal morphology or constituents were found between aminergic varicosities displaying synaptic contact and those lacking it. Thus, given the large number of profiles of axons and boutons studied, it seems likely that in this region the percentage of noradrenergic and serotoninergic varicosities making synaptic contact is substantially lower than that which occurs with nonaminergic neurons.

The results of these radioautographic studies in the neocortex contrast with the findings of a recent investigation of axon terminal ultrastructure in the dentate gyrus of the hippocampus (Koda et al. 1978). In this study the presence of small granular vesicles (SGV's) in boutons was used as the criterion for distinguishing noradrenergic terminals. Various treatments (drugs, lesions) that specifically lower hippocampal levels of norepinephrine produce correlated reductions in boutons containing SGV's. The evidence is good that noradrenergic boutons contain SGV's and that the preponderance of SGV-containing boutons in the rat dentate gyrus is noradrenergic, but it is not clear that all such boutons are noradrenergic. Koda and coworkers found that about 20% of boutons in the dentate gyrus, whether with or without SGV's, displayed synaptic junctions. The actual incidence of junctions is presumably higher, since random sections will not reveal all synapses. Thus, this study suggests a higher incidence of synaptic contact by noradrenergic varicosities than that found by Descarries and coworkers and does not reveal differences in incidence between noradrenergic and other boutons.

The difference between the findings of these two groups might be accounted for by factors such as:

1. Differences in the populations of boutons identified by the criteria of SGV-presence and ^{3}H-norepinephrine accumulation.

2. Regional differences in the incidence of synaptic contact. These EM studies, of course, can examine only a miniscule piece of tissue in the few regions so far examined in detail. Even though noradrenergic fibers examined in diverse parts of the brain may arise from the same cell bodies (especially those from the locus coeruleus), it does not necessarily follow that releasing sites in all branches must bear the same morphological relationship to their target neurons. Conceivably that relationship, particularly the distance between releasing sites and targets, depends on the organization and functions of the circuitry of the target tissue.

3. Occurrence of nonsynaptic boutons along axons that are not noradrenergic in some brain regions.

The procedures used for tagging aminergic boutons for EM identification can affect the apparent incidence of synaptic contact observed. For example, Tennyson and coworkers (1974) observed that only about 2% of dopamine boutons in the neostriatum exhibited synaptic junctions when tagging was accomplished by incubating tissue slices with 5-hydroxydopamine. In contrast, Arluison et al. (1978a) observed frequent synaptic contacts by dopamine boutons when 5-hydroxydopamine was injected *in vivo* and a different technique was used for fixation. It appears to be difficult to retain label in aminergic neuronal elements, and thus the distribution of label observed is affected by the concentration of label during incubation and by the technique used for fixation. Nevertheless, Arluison and coworkers also observed some dopamine boutons not displaying synaptic contact, and they concede the possibility of release of dopamine from some boutons synaptically (1978b).

Are serotonin and norepinephrine normally released from nonsynaptic boutons? There is no direct evidence on this point; indeed, it will be methodologically difficult to settle. However, there is good reason to ask. As Descarries and coworkers point out (1977), nonsynaptic varicosities appear to have all the apparatus, normally associated with release, found in varicosities making synapses. Furthermore, it would be surprising if release from the small fraction of varicosities demonstrating synaptic junctions could account for the amount of norepinephrine and serotonin that can be released by stimulation *in vivo* (Reader et al. 1976; Tanaka et al. 1976) or by depolarization *in vitro* (Dismukes & Mulder 1976, and refs. therein). In the spinal cord, substance P has been immunocytochemically visualized in extracellular space in patterns suggesting that it can be released from neurons in packets that diffuse to neighboring cells and capillaries (Chan-Palay & Palay 1977).

Some light may be shed on the question of nonsynaptic release of CNS transmitters by the much more thoroughly understood neuromuscular junctions of the peripheral nervous system (reviewed by Burnstock & Costa 1975). Junctions between noradrenergic boutons and effector cells in the periphery vary considerably, depending on the organ and type of cell. Junctional clefts range from 15–20 nm (vas deferens and iris) to 1000–2000 nm (large elastic arteries). In neuroeffector junctions whose membrane separation is greater than 20 nm, no postjunctional specialization is apparent. In closely apposed junctions, several sorts of postjunctional features have been described, including increased electron density. This, however, is rare, and Burnstock & Costa suggest (1975, p. 53) that these areas might represent mechanical attachment sites between cells rather than a measure of the region of transmitter action.

These findings raise questions about how synaptic contact is defined in the CNS. Should we consider close apposition of membranes with specialized features a sine qua non of chemical transmission between neurons? The morphology of synaptic junctions in the CNS is in fact diverse, and postsynaptic membrane specialization is not always apparent (Shepherd 1974, pp. 26–34). It might turn out that what appear to be nonsynaptic varicosities release amine neurotransmitters for diffusion to a single adjacent neuron whose receptors are not confined to a small postjunctional patch. This would allow the aminergic fiber to bias the largest cell's responses to its many synaptic inputs more effectively than would be possible with a single junctional contact. Such an arrangement would suggest a modulatory role, which is highly consistent with the character of the electrophysiological responses elicited by amine neurotransmitters (see discussion below).

The possibility of transmitter release from nonsynaptic boutons has been suggested by several authors as a basis for modes of communication in which transmitters released from a single site diffuse to multiple distant targets. If release into extracellular space did occur, there would be severe restrictions on information transmission in this mode. Cellular uptake and enzymatic degradation would sharply limit how far an amine or peptide transmitter could diffuse. Clearly, molecules released in such a fashion could not be used to transmit detailed temporal information about activity in the releasing neuron, because rapid onset and offset of pulses would be quickly obscured in diffusion over distance. Thus, transmission of slowly varying or tonic influence is suggested.

Chan-Palay has examined (1976) the extensive plexuses of serotinergic neurons, originating in raphé nuclei, which form supra- and subependymal systems in the walls of the cerebral ventricles. Identified in EM by radioautography, these serotinergic fibers contain varicosities with dense core "synaptic" vesicles. No evidence was found for specialized synaptic contact with ependymal cells or axonal processes. Chan-Palay has suggested (1977) that these fibers release serotonin into the cerebrospinal fluid (CSF), which would provide a means of transporting the transmitter to distributed targets. Although there is no direct evidence for such a function, it is not clear what purpose these fibers would serve if they did not release serotonin. Various authors have suggested that the ventricular system may be more than just a sewer. For example, Dunn (1978) recently proposed that the cerebrospinal fluid may be used to transport neuropeptides from release sites to distant target cells. He noted that neuropeptides are found in significant concentrations in ventricular CSF. Furthermore, proteolytic enzymes are notably lacking in the CSF, and intraventricular injection has proved an effective means of delivering exogenous peptides to brain receptors.

Direct evidence for nonsynaptic release of a peptide has been obtained in Aplysia. Neurosecretory bag cells of the abdominal ganglion to not appear to make synapses (Coggershall 1970). Their processes terminate in connective tissue, apparently releasing products directly into the hemolymph for diffusion within the ganglion and beyond. It appears that bag cells release several kinds of peptides, affecting the activity of various types of target cell in different locations (Blankenship 1979). For example, evoking spike activity in bag cells was found to produce characteristic responses in a nearby identified neuron, R15 (Branton et al. 1978). In contrast to conventional postsynaptic actions, these responses were slow in onset and persisted from minutes to hours. Direct application of bag cell extract (containing one or more neuropeptides) elicited identical responses in R15.

Neurotransmitter release is not limited to axonal varicosities and terminals. Dendrodendritic synaptic contacts have been reported in several brain regions (Shepherd 1974; Rakic 1975). Neurons originating in the substantia nigra apparently release dopamine not only from their distant axonal terminals but also from cell bodies and dendrites. Release can be stimulated by depolarization either *in vivo* (Nieloullon et al. 1977) or in slices (Geffen et al. 1976), and it is blocked by the removal of Ca^{++}.

Iversen and Cuello (Iversen 1979) found no evidence for synaptic vesicles or dendrodentritic contacts in nigral cell bodies and dendrites labelled by either ^{3}H-dopamine or 5-hydroxydopamine. Their EM studies suggested, rather, that dopamine is stored in cisterns of smooth endoplasmic reticulum. In contrast, Wilson et al. (1977), using markedly higher concentrations of 5-hydroxydopamine, observed the marker in conjunction with synaptic vesicles and found the vesicles in dendrites in synaptic contact with other dopaminergic dendrites. The different findings of these studies may reflect the failure of lower concentrations of 5-hydroxydopamine to effectively label vesicles in outlying dendritic tips. Also arguing for vesicular storage and release of dopamine is the dependence of release on Ca^{++}, which is thought to be involved in the interaction of vesicles with cell membrane in exocytosis.

Indirect evidence suggests that some dopamine released from nigral dendrites may diffuse to some targets nonsynaptically. GABA fibers from the striatum terminate in the substantia nigra and cause inhibition of cell firing therein (see Groves et al. 1975; Iversen 1979). These GABA terminals may be the site of presynaptic dopamine receptors coupled to adenylate cyclase that are observed in the substantia nigra (Spano et al. 1976; Gale et al. 1977). Dopamine at low concentrations has been found to specifically stimulate the release of ^{3}H-GABA from nigral terminals (Reubi et al. 1977). Iversen (1979) has proposed that dopamine released from dendrites in the substantia nigra may stimulate the release of GABA from terminals presynaptic to the dopaminergic cell bodies, thus providing a negative feedback loop. The presynaptic dopamine dendrites observed by Wilson and coworkers (1977) all appeared to be in contact with other dendrites, and whenever the postsynaptic element could be identified, it appeared to be dopaminergic also. No evidence was found for synaptic contacts between presynaptic dopaminergic dendrites and axon terminals. Thus, if the Iversen model is correct, dopamine released from dendrites may diffuse to GABA terminals that are not in direct synaptic contact. Similarly, Groves (1979) and coworkers (1975) have proposed a model in which dopamine released from dendrites produces self-inhibition of firing. This inhibition might be produced either by dendrodendritic synapses between adjacent neurons, by nonsynaptic diffusion of dopamine to autoreceptors, or by some combination of both.

In summary, nonsynaptic release of transmitter has not been clearly established in any central mammalian neuron other than those of the neuroendocrine system. However, a range of evidence from diverse systems suggests that it is an important possibility to consider and to attempt to test experimentally. Demonstration of nonsynaptic release would not necessarily be a revolutionary finding; in fact, it would fit rather nicely with the emerging concepts of the electrophysiological actions of amines and peptides that will be described below.

Intercellular transport of nontransmitter molecules

Neurons exchange molecules in a variety of ways besides chemical synaptic transmission. Several forms of nontransmitter exchange are briefly mentioned here for the purpose of illustration. Little is known about the functions of these processes (Smith & Kreutzberg 1976). Their nature may be primarily metabolic or trophic, but they may also provide a means of communication, on a time scale much slower

than that of synaptic transmission.

Several exogenous macromolecules (nerve growth factor, tetanus toxin, cholera toxin, certain lectins, etc.) have been shown to bind with high affinity to nerve terminals; binding is followed by uptake and rapid retrograde transport (Schwab & Thoenen 1977). In the case of tetanus toxin this transport is followed by migration to dendrites and transsynaptic transport to second-order neurons. Neurons and glia have been observed to exchange amino acids, proteins, nucleotides, and nerve growth factor (Smith 1978).

This profuse molecular interchange appears to inform the perikaryon of events occurring at the cells' distant projections. Although metabolic and trophic functions are probably involved, there also exist mechanisms by which molecules transported into the neuron could alter its electrical responsiveness and output. For example, steroid hormones, acting on intracellular receptors that apparently alter genetic expression, modulate phosphorylation of a cytosol protein (Liu & Greengard 1976). This protein may be the regulatory subunit of protein kinase; if so, it might provide a means of regulating membrane properties. Nerve growth factor taken up by adrenergic ganglion cell terminals is transported to the perikaryon, where it triggers a selective increase in tryosine hydroxylase, the rate-limiting enzyme for synthesizing norepinephrine, the cell's transmitter (Schwab & Thoenen 1977).

Certain heterologous nonneuronal cells in culture communicate via gap junctions (Lawrence et al. 1978). Hormones acting on receptors specific to one type of cell cause the heterologous cell to respond. This communication may be mediated by cyclic AMP passed across the gap junctions. Invertebrate neurons also possess gap junctions, which are thought to provide a means of synchronizing electrical activity; however, such junctions have been observed only rarely in mammalian CNS. It would be extremely interesting if neuronal gap junctions were also found to exchange second messengers.

How valid is Dale's Principle?

A widely accepted principle of neuronal operation is that the same transmitter substance is secreted from all branches of a neuron's terminals (Dale 1935). From time to time this principle has been questioned, but no clear-cut violation has been established (Burnstock 1976; Kandel 1976; Osborne 1979). However, the powerful techniques recently developed for cytochemical localization reveal a number of neuronal types that contain more than one substance thought to act as a transmitter. Radioenzymatic analysis of several identified neurons in Aplysia reveals the coexistence of serotonin, octopamine, and acetylcholine (Brownstein et al. 1974). (However, the microdissection technique employed has been criticized as subject to contamination from other cells; Osborne 1977). Somatostatin-like immunoreactivity has been reported in some noradrenergic neurons of the sympathetic nervous system (Hökfelt et al. 1977). In the CNS, cells identified as serotonergic on the basis of radioautography were found by immunocytochemical methods to contain substance P (Chan-Palay et al. 1977).

The fact that a neuron contains some amount of several transmitter substances does not, of course, prove that each substance is being released as a transmitter. However, the giant cerebral neuron of Helix does appear to release both acetylcholine and serotonin, each of which produces postsynaptic responses (Cottrell 1977).

Sympathetic neurons cloned in tissue culture can readily be caused to form either adrenergic or cholinergic functional synapses by a choice of appropriate media conditions (Reichardt & Patterson 1977). Moreover, some microcultures containing only a single neuron have been found to secrete both norepinephrine and acetylcholine. Whether this also occurs in mature cells *in vivo* is not known, but it clearly indicates that there is no intrinsic biochemical or genetic reason why a neuron cannot simultaneously manufacture and release more than one transmitter.

There is an intriguing possibility that some neurons might release two kinds of molecules from the same terminal – one serving way to modify the action of the primary transmitter. For example, ATP is released along with catecholamines from the adrenal gland, and there is evidence that some ATP is released concurrently with norepinephrine from sympathetic fibers (Kopin 1967; Burnstock 1976). ATP has been proposed as a primary transmitter in certain peripheral organs (Burnstock 1975), but there is also a suggestion that after depletion of norepinephrine a continuing substantial noncholinergic, nonadrenergic response in the cat nictitating membrane may be due to release of ATP from the adrenergic nerves (Langer & Pinto 1976).

There is evidence that adenosine can be released from central neurons, can stimulate cyclic AMP formation through specific receptors, and can alter postsynaptic electrical activity (see Fox & Kelley 1978). Schubert and coworkers (1976) have suggested that adenosine or its derivatives may function as a "secondary" or "additional" transmitter producing long-lasting alterations in target-cell activity. However, there is not yet any direct evidence that these nucleotides are released in conjunction with a primary transmitter.

It has been proposed that minute amounts of amines such as para-tyramine and phenylethylamine, released along with norepinephrine from adrenergic fibers, act as neuromodulators rather than as neurotransmitters (Boulton 1976 and refs. therein). However, the distinction between "neurotransmitter" and "neuromodulator" is problematic (see discussion below), and it may be better, as Burnstock (1976) suggests, to retain in the category of transmitter any substance produced and physiologically released from nerve terminals to evoke postsynaptic responses through membrane receptors.

Dale's Principle is not invalidated by these findings. Given that there are very few systems in which we can determine the transmitter released from each branch of a neuron, it remains a useful operating assumption. However, the principle has at times been interpreted dogmatically, as an invariant rule, although this was never intended by Dale himself (1935). The danger of overextending general working principles is that the exploration of important exceptions and variations is discouraged.

Complex electrophysiological responses to neurotransmitters

Integration of synaptic inputs is a central feature of neuronal operation. Early concepts portrayed the receptive portion of the neuron as a linear integrator, adding up excitatory and inhibitory inputs to evoke an output – firing of impulses – proportional to the sum of inputs. Later investigations, however, revealed that postsynaptic integration is much more complex, involving a number of distinct processes (Bullock 1959; Rall 1970; Shepherd 1974). Synaptically evoked dendritic potentials spread passively, for the most part, declining in magnitude as they spread, because of resistance losses. (Some dendrites, however, are capable of active conduction; Shepherd 1974.) Thus their ability to influence action-potential-generating sites in the cell body depends on geometric relationships, and in neurons with extensively branching dendritic fields (e.g. the cerebellar Purkinje cell) those relations can be quite complex. Further complexity is added by the occurrence in some neurons of patches of excitable dendritic membrane which by interaction with nearby simultaneously active dendritic synapses, alter resistance of the membrane through which passive potentials must pass.

In recent years considerable evidence has accumulated for the occurrence of postsynaptic potentials that do not fit neatly into either excitatory or inhibitory categories. It has been suggested that the function of these potentials is to modulate the effectiveness of excitatory and inhibitory synpatic inputs rather than directly influencing firing of the postsynaptic neuron.

The excitation or inhibition produced by most synaptic junctions can be explained in terms of increases in the permeability of the postsynaptic membrane. The difference in resting potential across the membrane is caused by differential permeability to ions, permeability for Na^+ being much less than that for K^+ or Cl^- (Katz 1966). Decreasing the difference in permeability depolarizes the membrane; increasing it causes hyperpolarization. In principle,

either depolarization and hyperpolarization can be accomplished by increasing conductance for one set of ions selectively, or by decreasing conductance for another set.

In most cases excitatory postsynaptic potentials (EPSP's) and inhibitory postsynaptic potentials (IPSP's) are created by selectively opening membrane channels for one or more ions. However, in certain neurons, slow postsynaptic potentials have been observed (Koketsu 1969; Libet 1970) that do not appear to involve opening of channels, for they are accompanied by *increases* or no change in membrane resistance (the reciprocal of conductance). Slow EPSP's and slow IPSP's in some sympathetic ganglion cells apparently result from decreased conductance by specific ion channels (Weight 1974), although other mechanisms may also be involved (Kobayashi & Libet 1974).

Schulman & Weight (1976) have proposed that slow potentials in which membrane conductance is decreased (and hence the resistance is increased) may provide a mechanism for modulating neurotransmission by varying the effectiveness of fast PSP's generated by conventional synaptic inputs. When membrane resistance is increased, postsynaptic dendritic potentials will be less attentuated, due to shunting, and thus can spread further. Neurotransmitter receptors that elicited increases in resistance over wide patches of postysynaptic membrane could thus modulate the ability of synaptic inputs to influence postsynaptic firing by altering the spread of fast PSP's toward the cell-body site that generates action potentials. Evidence for this sort of mechanism was obtained in bullfrog sympathetic ganglion cells in which activity in presynaptic fibers elicits both fast and slow EPSP's (Schulman & Weight 1976). Production of slow EPSP's was found to increase the amplitude of fast EPSP's elicited by presynaptic activity and to enhance greatly the ability of these fast EPSP's to elicit postsynaptic firing. This potentiation lasted several minutes.

Decreases in membrane conductance during synaptic or pharmacologic activation of some slow potentials have been reported for some central neurons (Krnjevic et al. 1971; Siggins et al. 1971). The effects of norepinephrine on central neurons are generally described as inhibitory, but recent studies show a complex mode of action (Freedman et al. 1977). Norepinephrine inhibits the spontaneous firing of cerebellar Purkinje cells but enhances the fast EPSP's evoked by climbing fibers and the fast IPSP's induced by basket- and stellate-cell inputs. The enhancement of convergent inputs persists for some minutes after spontaneous activity returns to normal. These results may be explained by the observation that norepinephrine increases Purkinje cell membrane resistance. Thus, noradrenergic input (arising from the locus coeruleus) may alter the Purkinje cell's mode of operation, switching emphasis from one set of inputs to another. Such a modulatory role would be consistent with the anatomical considerations discussed in the early part of this paper.

The suggestion that some transmitters can modulate the efficacy of transmission of other synapses is intriguing and may have important implications for the ways in which neurons process information and even for the mechanisms of learning and memory. However, the evidence is as yet incomplete, and a number of questions will have to be dealt with to evaluate this possibility. For example, one might point out that conventional postsynaptic potentials must also alter (restrict) the spread of potentials from convergent synapses by decreasing membrane resistance in the immediate area. In that sense activity at any synapse could be said to modulate the efficacy of other nearby synapses. However, this argument would be countered if it could be shown that the conductance decreases elicited by certain inputs are spread widely over the postsynaptic membrane. This mechanism could then influence the efficacy of large numbers of synaptic inputs, whereas conventional responses would effect only immediately adjacent synapses. This is in fact quite plausible, since slow PSP's are generally thought to be mediated by second messengers (Greengard 1976, but see also Phillis 1977) such as cyclic nucleotides, which could produce membrane changes in almost any part of the cell.

Another question concerns the possibility that the slow IPSP's would produce counteracting effects – for example, a decrease in postsynaptic firing due to membrane hyperpolarization, and an increase in firing because of enhancement of convergent EPSP's. Which effect would predominate would of course depend on the geometry of synapses in the dendritic field, and whether the conductance increases were in fact widely spread by a second messenger. At this stage it might be wise to avoid trying to pin any exclusive label on the function(s) served by these complex electrophysiological responses.

Neuropeptides may act as conventional neurotransmitters in some neurons (Iversen et al. 1978). However, a number of reports have described electrophysiological responses to peptides that are more complex than conventional excitation or inhibition. For example, Zieglgänsberger & Bayerl (1976), recording from spinal neurons *in vivo*, discovered that opiate agonists both depressed spontaneous activity and blocked excitations produced by glutamate and acetylcholine. These effects of opiates were probably mediated by receptors for endogenous peptides, since they were blocked by specific antagonists. (Similar effects have recently been reported for met-enkephalin by Denavit-Saubié et al. 1978.) The mechanism of this opiate action is not known, but it does not appear to be due to hyperpolarization, since the opiates produced no observable change in membrane potential or resistance. (However, it is hard to eliminate the possibility that in some distal dendritic regions changes in membrane potential or resistance occurred that would not be detected in recordings from the cell body.)

Neuropeptides produce diverse effects in invertebrates (Barker 1978). In addition to simple excitation and inhibition, certain neuropeptides elicit responses that are unusual in that: (1) they have a prolonged time course (up to several hours), and (2) they appear to involve not just the voltage-independent changes in conductance conventionally associated with postsynaptic responses, but also voltage-dependent conductances of the type underlying the generation of action potentials and pacemaker potentials. For example, vasopressin was found to induce a bursting pattern of firing in an identified neuron that had not shown bursting in the absence of the peptide (Barker & Gainer 1974). This was apparently accomplished by switching the current-voltage relationships of the neuron from a linear form to a nonlinear form characteristic of bursting cells.

Complex effects of peptides have been examined in detail in cultured spinal neurons (Barker 1978). Substance P and leu-enkephalin were found to produce three forms of unusual response in addition to simple excitation and inhibition: (1) elevation of thresholds for spike generation, (2) abrupt depolarizations not involving normal activation of membrane conductances, and (3) modulation of the amplitude of voltage and current responses to glycine and glutamate, without directly altering membrane polarization or resistance. The physiological significance of these findings is not certain, since it is not clear what changes may have occurred in the neurons in culture, or whether they would ever be exposed to peptide transmitters *in vivo*. Nevertheless, this is clearly an important model, particularly since the responses observed resemble to some degree those occurring in spinal neurons *in vivo* (where peptide transmission clearly is involved).

These studies taken together indicate that some neurotransmitters produce in some neurons rather complicated responses that are not simply excitation or inhibition, as conventionally defined. The extent of occurrence and the function of these complex actions remain to be established. Nevertheless it is worth noting that the effective consequence of these nonconventional actions seems to be a switching of the mode of operation of the target neuron. In some cases this appears to involve a change in the way the neuron integrates its conventional synaptic inputs.

What is a modulator?

The words "modulation" and "modulator" have appeared in the titles of several hundred neuroscience articles in the past year (according to an informal computer survey of the literature). Modulation is being used increasingly to characterize actions of putative

transmitters that do not seem to fit within traditional concepts of neuronal action. By extension, it has been suggested that endogenous substances that elicit these unconventional responses – particularly monoamines and neuropeptides – might be considered neuromodulators or neuroregulators rather than neurotransmitters.

The concept of modulation has not been applied in any consistent way, and explicit definitions have seldom been attempted. Modulatory roles have been ascribed to various neurochemical systems on the basis of diverse characteristics, such as the nature of the electrophysiological response, the time course of action, the mode of transmission to target cells, and the distribution of targets. This ambiguity is heightened by use of the word in different contexts to describe actions at different levels of organization. Besides cellular actions, modulation has been applied at the circuit level to describe gating of motor outputs, and at the behavioral level to refer to changes in response strength that might be produced by arousal, motivation, or learning (Kandel et al. 1979). It is intriguing to speculate that modulation of behavioral function, arousal, for example, might be mediated by neurochemical systems such as the diffuse ascending aminergic pathways, producing modulation at the cellular level. Unfortunately there is still little evidence for such confluence, nor is there likely to be more until our notions of modulation can be stated more explicitly.

In one of the few explicit definitions that has been made, Florey (1967) suggested that "modulator substance" be used " . . . for any compound of cellular and nonsynaptic origin that effects the excitability of nerve cells . . ." and that ". . . can effect the responsiveness of nerve cells to transsynaptic actions . . . and can alter . . . spontaneous activity." Barchas and coworkers (1978) distinguished neuromodulators (neuroregulators) from neurotransmitters as compounds that are important in general communication between nerve cells, but which operate in a hormone-like fashion, rather than transsynaptically. (This definition is similar to Chan-Palay's (1977) characterization of "neurohumors;" see following section.)

Unfortunately, using nonsynaptic transmission as the primary defining characteristic fails to draw together all classes of action that might be considered modulation, and furthermore it creates some awkward divisions. For example, would the lack of close synaptic apposition at most noradrenergic boutons *en passant* in the peripheral nervous sytem force us to call norepinephrine a neuromodulator rather than a neurotransmitter in these regions? Would modulation of transmitter release by synapses on nerve terminals not be considered the work of a modulator, since the modulating compound was released synaptically? Would the complex electrophysiological effects described in the previous section not be considered an example of modulation, unless the monoamines and peptides evoking them were shown to be released nonsynaptically? The difficulties raised by these questions suggest that defining the mode of transmission does not necessarily distinguish the nature of modulatory actions. The possibility of distinct modes of transmission will be considered further in the next section.

Given the incomplete state of our knowledge, I suggest that there is little advantage at this time in attempting to distinguish modulators from transmitters. On the other hand, there may be some heuristic value in trying to clarify what *actions* might be considered to be modulatory. The various uses of the word modulation seem to have in common mainly the general English language connotation of alteration of a primary characteristic over time by a secondary influence. At the cellular level most usages imply that a modulator changes the efficacy of conventional synapses without directly altering the rate of firing or release of transmitter. Presynaptic modulation of transmitter release fits this notion neatly. The release of transmitter is clearly caused by axonal impulse activity and is only modified by presynaptic receptors. Postsynaptically the situation is more ambiguous. Most central neurons receive many synaptic inputs, and it is seldom possible to say that any one exerts primary control. As was previously pointed out, a conventional synaptic excitation or inhibition can modify the effectiveness of nearby synapses. However, it is plausible that some transmitters produce responses whose predominant effect is alteration of other inputs. Such transmitters might be released either synaptically or nonsynaptically and could act on nerve terminals, dendrites, or cell bodies.

When it appears that the predominant effect of a transmitter substance in a particular system is regulation of the efficacy of other transmitter inputs or of the mode of operation (e.g. bursting vs. nonbursting) of the target cell, then it may be useful to think of this action in terms of modulation. I suggest that is is preferable to use the word modulation as a broad, generic term at present, rather than trying to pin it down to a particular function, because we are just beginning to discover the range of responses that transmitters can evoke in various neurons. It may turn out that an array of functions beyond simple excitation and inhibition will appear. Furthermore, it would be well not to label particular transmitters as neuroregulators or neuromodulators unless the specific system is identified, because a given agent might evoke various responses in different target cells. The character of response is, of course, not a function of the transmitter molecule itself but of the effector mechanisms to which its receptors are coupled.

Are there distinct modes of chemical communication?

Sharrer pointed out a decade ago (1969) that the borderline between the two well-established classes of neural mediation – classical synaptic transmission and neurosecretion of hormones – is less sharp than had been previously thought, in that some neurons demonstrate characteristics overlapping both classes. Chan-Palay has suggested (1977) that the nonsynaptic varicosities of serotonergic and noradrenergic axons are the basis of a specific mode of neuronal transmission, which she terms neurohumoral. This mode is conceived to be intermediate between classical synaptic transmission and hormonal secretion. Neurohumors released from diffuse aminergic axonal projections could alter the responsiveness of vast domains of target cells, possibly constituting a way of modulating the behavioral state of the organism. The serotonin fibers lining the walls of the cerebral ventricles provide a striking illustration of this possibility. Serotonin released into the cerebrospinal fluid (CSF) might have access to a wide range of targets, either by re-uptake and transport by certain ependymal cells lining the ventricles, or by the flow of CSF from the ventricles over the cortex. By modulating the activity of widespread target neurons, serotonin might thus have global effects on behavioral state.

Barker (1977; 1978) has proposed a similar mode of communication, termed neurohormonal, on the basis of observations with neuropeptides. Neurohormonal communication was characterized as nonsynaptic, acting by regulation of voltage-dependent spike or pacemaker conductances. In contrast to synaptic moment-to-moment regulation of single-neuron excitability, it would provide sustained regulation of neuronal aggregates, perhaps coordinating their output. Barker (1978) also proposes another mode of communication – "neuromodulation" – which operates as a form of gain control over transmission through conventional synapses. Neuromodulation was proposed to operate through alteration of synaptically activated, voltage-independent conductances and might or might not be restricted to continguous cells. By defining neuro-modulation explicitly in terms of electrophysiological phenomena, Barker has avoided the problem of ambiguity discussed in the previous section. However there might be some advantage to coining a new term for this specific electrophysiological action, to avoid confusion with all the other uses of the word "modulation".

These proposals have heuristic appeal because they draw together a number of striking features of monoamine and neuropeptide presumptive transmitters in terms of their possible functional significance. Although it is highly speculative, this provides a conceptual framework for thinking about features as diverse as: the diffuse anatomy of aminergic pathways, the slow monotonic pattern of firing in those neurons, the possibility of nonsynaptic release, and complex electrophysiological responses. At present, evidence is too fragmentary to evaluate how well these proposed categories of transmission might accommodate emerging data about the diverse

ways in which neurons communicate.

Given this state of affairs, it is important to maintain a flexible approach to categorizing neuronal communication. Bloom has proposed (1979) such an approach, in which transmitters would be classified in terms of three domains of action: time course, spatial distribution, and energy. This last domain seems ambiguous but apparently concerns the character of the response elicited by the transmitter. This scheme may be useful, for it allows organization of emerging data without forcing premature labeling. As the growing number of putative transmitters are plotted on this three-dimensional array, certain groupings that correspond to distinct modes of transmission may become apparent.

Nomenclature for complex actions of transmitters and for proposed modes of transmission is becoming chaotic. Substances have been distinguished as neurotransmitters, neurohumors, neurohormones, neuromodulators, neuroregulators, and neuromediators by various authors in ways that are sometimes overlapping, sometimes conflicting. Confusion might be avoided if, at this stage, labels were avoided and distinctions were spelled out – for example: nonsynaptic vs. synaptic release, excitation via opening of voltage-independent ion channels vs. activation of voltage-dependent conductances. However, in practice this would be tedious, and it seems unavoidable to use labels. I suggest that the following approach to nomenclature would be reasonably consistent with conventional definitions found in technical dictionaries (e.g., Blakiston's 1972) and would allow a desirable flexibility:

Neurohumor. a generic term for any substance released by a neuron to alter the activity of other cells, adjacent or distant.

Synaptic (or junctional) neurotransmitter. a neurohumor that is released for diffusion to an adjacent excitable cell (and to autoreceptors, when present). The junction may be broader than that found in Grey's Types I and II synapses, and specialization of the postsynaptic membrane may not be apparent (Shepherd 1974). The response to a neurotransmitter, synaptic or nonsynaptic, is mediated through membrane receptors and may include complex modulatory actions as well as conventional excitation or inhibition.

Nonsynaptic (or nonjunctional) transmitter. a neurohumor that is released for diffusion or transport to multiple excitable cells, which may be distant from the release site.

Neurohormone. a neurohumor capable of regulating mulitple and distant target cells that do not necessarily have membrane receptors or electrical responses. Transport is primarily via vascular channels.

Obviously additional terms will be necessary if other classes of neurohumors or subclasses of nonsynaptic transmitters are delineated. In that case it would be desirable to coin new words to avoid confusion with old terminology. Interestingly, the classic criteria for identifying a substance as a central neurotransmitter, as formulated by Werman (1966), do not require synaptic action and thus could be applied to other neurohumors as well. Some other statements of the criteria, however, do explicitly describe synaptic transmission. For example, Barchas and coworkers (1978) distinguished neuroregulators as operating nonsynaptically and proposed a modified set of criteria for them.

Some implications and conclusions

It remains to be demonstrated that amine-containing boutons without apparent synaptic contact release their transmitter *in vivo*. If such release does occur, the transmitter might diffuse to multiple target cells, but alternately it might activate only an immediately adjacent neuron, perhaps through receptors spread more broadly over the membrane than would occur in a synaptic junction. Thus the form of release of transmitter from these diffusely-branching aminergic fibers in the brain may be analogous to that found in peripheral noradrenergic fibers. The possibility that fibers lining the walls of the ventricles might release serotonin in the CSF for transport to distant targets is much more novel and would seem to represent a mode intermediate between synaptic and hormonal transmission.

If nonsynaptic transmission does occur, it would not necessarily require any radical revision of our conceptualization of neuronal function, but rather it would seem to complement known features of the diffuse ascending aminergic pathways. For example, the noradrenergic fiber system projecting from the locus coeruleus throughout many regions of the brain has several striking features:

1. Projections so diffuse that one noradrenergic neuron may influence vast numbers of target cells.
2. A slow, monotonic pattern of impulse activity that seldom varies.
3. Production of target-cell responses that are long in latency and duration and that modify the effects of other transmitters.

Such a system seems unlikely to transmit detailed information about rapidly-changing processes, but rather it is generally conceived to act in some regulatory fashion, modulating the level or pattern of responsiveness of its targets.

Both Chan-Palay (1977) and Barker (1977, 1978), in proposing nonsynaptic modes of transmission, have suggested that these might serve as a means of coordinating or tuning the activities of large arrays of target neurons. Modulating the functional state of neuronal ensembles could be a way of regulating the state of behavioral responsiveness of the organism (Hobson & Scheibel 1979). This sort of regulation might underlie functions such as: gating of sensory information in intermediate processing centers such as the thalamus, sleep/wakefulness cycles, arousal, reinforcement of behavior, memory formation, and affective state.

Over the past two decades monoamine transmitters have been implicated in many aspects of animal behavior and of psychiatric disorder (Lipton et al. 1978), and recently there have been many similar suggestions about neuropeptides (Liebeskind & Dismukes 1978). However, only in a very few cases has the actual nature of the transmitter's involvement been established. Modulation of the functional state of neuronal ensembles is a heuristic concept that may be very useful in understanding mechanisms underlying behavior. Hints of behavioral involvement have been observed much more often with monoamines and peptides than with transmitters such as acetylcholine or GABA. This might be an indication that experimental manipulations of transmitters involved in state regulation of neuronal ensembles produce behavioral manifestations more concordent than would occur with manipulation of an excitatory or inhibitory transmitter involved in diverse circuits. It should be noted that the argument for some transmitters acting as regulators of functional state is based primarily on the nature of their electrophysiological action and on behavioral inferences. Nonsynaptic transmission would not necessarily be required, although it would extend the range of action.

The studies briefly reviewed in this article suggest several aspects of neuronal communication not encompassed by conventional descriptions. This does not mean that traditionally stated principles of neuronal operation are wrong, but rather that they are incomplete and perhaps overgeneralized. Current research suggests that an array of information-processing mechanisms overlie well-known central themes. Thus, for example, the concept of integration of excitatory and inhibitory synaptic inputs is not invalidated by evidence that the summation, rather than being a simple algebraic addition, is instead a complex and dynamic interaction that includes various regulatory and adaptive processes. In a similar fashion we still talk of dopaminergic neurons transmitting information from the substantia nigra to the striatum as a primary theme, even though it is overlaid with secondary actions such as dendritic release of dopamine in the nigra, feedback inhibition at autoreceptors on striatel terminals, and perhaps retrograde transport of macromolecules.

What is invalidated by these various lines of research is any notion of the neuron as a black box, analogous to an electronic operational amplifier, which can be described as performing a set transformation on its inputs to produce a corresponding output. Clearly, neurons are pretty sophisticated little computers in their own right, with mulitple

layers and time scales of information processing. Their modes of interaction may eventually prove as diverse as their anatomical forms.

Successful explanations in biology sometimes become overgeneralized as they are extened to new systems and the original experimental qualifications are forgotten. The traditionally stated principles of neuron operation have provided a framework for understanding a wide range of phenomena, but the success of these central themes should not reduce our sensitivity to additional forms of molecular exchange and information processing among neurons. It is important not to let working principles turn into dogma, or to replace old dogmas with new ones. Ironically, one of the most generalized of conventional postulates is named after Sir Henry Dale, who apparently intended his principle to be considered only as a guide (Dale 1935; Burnstock 1976).

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NOTE

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by **S. Arch**

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Terminology, modes of communication, and a command neurohormone. The value of the concept of alternative modes of communication among neurons is apparent only if it leads to a better understanding of the normal physiology of the organism. In one of the more thoroughly studied preparations, this understanding seems to be forthcoming. The bag cell neurons in *Aplysia californica* synthesize and secrete a polypeptide hormone that causes a complex egg-laying response in the animal (reviews: Arch 1976; Blankenship 1979). The egg-laying hormone (ELH) acts on the gonad and other portions of the reproductive tract to cause changes in muscular activity, and on the heart to accelerate and strengthen beating. Moreover, ELH is known to cause a marked change in the behavior of the animal. Thus its effects in the nervous system are of considerable interest. Perhaps the most striking feature of ELH action in the nervous system is the large number of cells it appears to influence. While most of the effects remain to be shown as direct – and many may not be – ELH action results in a significant alteration of central nervous system activity.

The sum of the hormone's effect is to cause the animal to lay its eggs in the species-typical fashion, and each of the known actions of ELH can be interpreted as serving this end. Thus, in a sense, the hormone seizes control of the animal's physiology and directs it to perform this reproductive function. For this reason I believe that it should be considered a *command neurohormone*. It is both necessary and sufficient (Kupfermann and Weiss 1978) for the initiation and organization of the egg-laying response.

It might seem less than fully responsive to the spirit of Dismukes's article to propose still another term to characterize the functional role of a substance active in the nervous system. I am, in fact, in agreement with Dismukes on the point that we should be explicit about the synaptic, nonsynaptic, or neuorhormonal nature of the influence we are examining. In some respects, however, the development of an "official" terminology may be either a waste of effort or, worse, a hindrance to understanding. As we learn more about the physiology of the various systems under active investigation, our terminology may become obsolete. More dangerous is the fact that annointing some terms and proscribing others may impose a dogmatic control over the way we view our results. The problem that should concern us right now is not that of terminology, but that of demonstrating the existence of alternative modes of behaviorally relevant neural intercommunication. For this task we need a set of criteria, whose satisfaction will permit the conclusion that an alternate channel of communication has been identified.

Accumulations of vesicles near cell membranes are not necessarily indications of secretion. Identification of a suspect compound in a cell or brain region tells us nothing about its action (if any). Neural response to an exogenously applied substance is not a demonstration of its significance in normal physiology. Such observations may be suggestive, but they permit only speculation. We will not be in a position to conclude that alternative modes of communication exist until we have satisfied essentially the same set of criteria that we recognize for the identification of a synaptic neurotransmitter. Indeed, we probably need to add at least one criterion to the list. Since our concern is with substances that may be released nonsynaptically and may act on extrasynaptic sites, the route taken by the substance becomes important. Specifically, it should be shown that the putative neuroactive compound can traverse the barriers, if any, between its release site and the structures upon which it is supposed to act.

In the *Aplysia* system I believe we are close to satisfying these criteria. To call ELH a *command neurohormone* then, is simply a way of characterizing its role economically. Other substances in other systems have been, and will be, characterized with other words. The only chaos in our literature that need concern us will come not from too many functional names for neuroactive compounds, but from too little empirical support for the functions implied by the names.

by **M. Arluison**

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The problem of nonsynaptic transmission in the neostriatum. This commentary examines the problem of the occurence of nonsynaptic dopaminergic transmission in the nucleus caudatus-putamen (NCP). In this line of work, our results, dealing with the description of dopaminergic contacts in NCP (Arluison et al. 1978a) conflict with those of Tennyson et al. (1974), which strongly supports the existence of nonsynaptic release. I can present only a brief commentary upon the discrepancy between the results:

1. In both studies, 5-OH-DA (5-hydroxydopamine) was used in rather high concentrations. In our work (Arluison et al. 1978a), the tagging of synaptic vesicles in nerve endings was studied according to the gradient of diffusion of the marker around the injection site and was controlled by destruction of the substantia nigra by 6-OH-DA. We found that an important nonspecific labelling of nerve endings occurred near the injection site because of the excess concentration. Such nonspecific capture probably also occurs in the work of Tennyson et al., in which 5×10^{-4} M of 5-OH-DA is used.

2. In our own two studies (using, respectively, 5-OH-DA or radioautography after administration of ^{3}H-DA), we tried, at first to quantify the presence or absence of labelling in certain nerve endings, studying only those terminals exhibiting differentiated synaptic contacts. In addition, we tried to see whether synaptic contacts were more numerous in the labelled population of nerve endings than in the nonlabelled one. Using 5-OH-DA, we found that the occurence of synapses was approximately the same in both groups. In contrast, with ^{3}H-DA we found that the percentage of labelled nerve endings was higher in the group of nerve endings without synapses.

3. In the studies of Tennyson and coworkers, tissues are incubated in 5-OH-DA, and this procedure alters the tissue such that at least certain synapses are destroyed. Moreover, fixation of tissue by $KMnO_4$ is not well suited for the identification of synapses because postsynaptic thickening is not visible. Under these conditions, the reported absence of synaptic contacts for dopaminergic fibres is understandable.

In conclusion, we agree with the possibility of the existence of

nonsynaptic transmission in the brain for monoaminergic fibres, but such a mechanism remains to be demonstrated in NCP.

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Intercellular communication in the CNS. Changes in neuronal excitability and the communication of excitability changes among neurons are considered crucial to the function of the nervous system. Intercellular communication between excitable elements has been studied in a variety of relatively simple invertebrate and vertebrate preparations. The application of increasingly rigorous techniques to these preparations has greatly increased our understanding of certain forms of intercellular communication. Thus, for example, we now have considerable understanding of the sequence of steps and the nature of the communicated signal at various neuromuscular junctions. The signalling takes place at specialized interfaces between the contiguous nerve and muscle elements called synapses and is known as synaptic transmission. Such communication has both anatomical and electrophysiological or biophysical definition.

The accessibility of various neuromuscular and invertebrate junctions has allowed resolution of the sequence of events occurring when changes in excitability in the "presynaptic" element are transformed into changes in membrane excitability of the "postsynaptic" element. Synaptic transmission involves a reaction between the transmitted molecule and receptor sites on the target cell, which alters membrane conductance of the target cell to specific ions. This effectively changes membrane potential and/or conductance. The membrane reaction takes place largely independently of the membrane's potential (see Katz 1966). Synaptic events are characterized as "inhibitory" or "excitatory" depending on whether the change in membrane potential and conductance produced leads in turn to activation of the conductances underlying action potential generation. Activation of the latter is highly dependent on membrane potential, unlike the membrane reaction associated with synaptic events. Functionally, synaptic signals produce a brief change in the excitability of the target cell.

Because communication between contiguous elements has been the most commonly and thoroughly studied form of intercellular communication, it has generally been assumed that it is the only form of communication and that all changes in neuronal excitability in the nervous system are the result of synaptic transmission. This assumption underlies the conclusion, so often expressed, that high-affinity binding sites for endogenous substances are evidence of receptors for neurotransmitters. At this point it is uncertain whether interneuronal communication in the central nervous system is solely and simply mediated by synaptic transmission as defined at peripheral synapses.

Although the geometry of individual cells and their anatomical relationship with each other are important considerations in understanding modes of intercellular communication, resolution of the physiological aspects of the signals communicated is a prerequisite to specifying what functional roles are involved in the communication. The evolution of new strategies and the advent of new preparations has led to observations of anatomical arrangements and functional effects of endogenous substances that are distinctly different from the conventional neurotransmission recorded in detail at neuromuscular junctions. These observations have prompted suggestions that the central nervous system needs an array of signals because the behavioral repertoire it can elaborate is far more complex than simple neuromuscular transmission and muscular contraction, and that while interneuronal communication certainly involves the transmission of information, this does not necessarily mean that all forms of communication are "neurotransmission."

I have attempted to summarize and categorize many of the observations relevent to our understanding of intercellular communication in the nervous system in Figure 1. All of the intercellular events in the first row (A-G) take place between contiguous elements. Electrical and synaptic transmission have well-established anatomical substrates. The former involves the simple and immediate transfer of charge through a low-resistance pathway between two cells. The latter involves the diffusion of substrates over short distances and the activation of conductance changes through the engagement of receptors. Geometry of receptor distribution, species of ionic conductance, and coöperativity of binding, as well as previous history of use are factors known to shape the synaptic signal. The events schematized in C and D involve alteration in voltage-dependent pacemaker conductances following activation of cholinergic synaptic inputs. Since these signals involve alterations in receptor-coupled, *voltage-dependent* conductances, they are operationally distinct from conventional synaptic transmission, which involves alterations in membrane conductance independent of voltage.

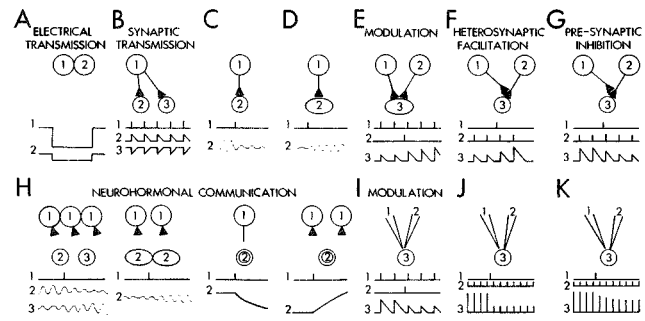

Figure 1. (Barker). Schematic diagrams of physiological and pharmacological signals relevant to intercellular communication in the nervous system. In each diagram the anatomical relationship of the communicating elements is depicted above, and the changes in electrical and chemical excitability occurring in the cells is shown below. Individual cells and their respective membrane potentials are numbered accordingly. Nerve cells are circular; smooth or cardiac muscle cells are elliptical, and vascular smooth muscle cells are shown as concentric circles. Filled triangles represent terminal regions releasing neuronchemicals. Micropipettes for artificial release of substances are illustrated in I-K.

A-G represent physiologically-elaborated signals occurring between contiguous elements. H gives examples of signals recorded between noncontiguous cells. I-K are pharmacological actions of endogenous substances which are functionally distinct from conventional neurotransmitter action.

A and B: For review, see Brookhart and Mountcastle (1977).

C: Cholinergic inactivation of pacemaker conductance in *Aplysia* neurons (Wilson and Wachtel 1978). Muscarinic inactivation of spike conductances of amphibian sympathetic ganglion cells (Kuba and Koketsu 1976).

D: Cholinergic activation of pacemaker conductance in smooth muscle (Bolton 1975).

E: Serotoninergic modulation of buccal contraction in *Aplysia* (Weiss, Cohen, and Kupfermann 1978).

F and G: See Kandel and Tauc (1965), Shimahara and Tauc (1975), and Klein and Kandel (1978) for demonstration of these events in *Aplysia*.

H: Bag cell peptide (Branton, Mayeri, Brownell, and Simon 1978; Branton, Arch, Smock, and Mayeri 1978; Brownell and Mayeri 1979) and vasopressin alteration of pacemaker and nontransmitter conductances on *Aplysia* neurons (Barker and Gainer 1974; Barker and Smith 1976; 1977). Adrenal medullary cells release epinephrine to alter pacemaker conductances in cardiac cells (Tsien 1974). Substance P mediation of vasodilatory reflex (F. Lembeck et al., unpublished observations). Vasoconstriction mediated by vasopressin released from supraoptic neurosecretory cells (see Gainer 1977).

I: Peptide (Barker, Neale, Smith, and MacDonald 1978; Vincent and Barker 1979) and dopamine (Libet and Tosaka 1970) modulation of transmitter events.

J: Peptide elevation of spike threshold (Barker, Gruol, Huang, Neale, and Smith 1978).

K: Depression of spike Ca^{++} conductance by peptides, amino acids, and catecholamines (Dunlap and Fischbach 1978; Mudge, Leeman, and Fischbach 1978).

In each case, molecules released from one modulate the efficacy of signalling between the other two elements, apparently by altering the level of intracellular Ca^{++}. In E the modulation occurs at the postsynaptic cell involved in the synaptic transmission, while in F and G the "facilitation" and "inhibition" of the transmission refer to changes in the efficacy of transmitter release from presynaptic terminals. These modulatory events, all of which have been described physiologically, fall outside the definition of conventional synaptic transmission and have thus received identifying labels.

It should be noted that the anatomical relations of the cells communicating these signals are not known with any degree of precision,

although the cells appear to be contiguous. Certain signals are communicated between noncontiguous cells via extra synaptic avenues, as schematized in the four examples shown in section H. The first has been described between nerve cells in invertebrate ganglia. In this example, peptide substances can alter the excitability of diverse and distant target cells by acting on both voltage-dependent spike and pacemaker conductances and voltage-independent (synaptic transmitter-like) conductances. The other three are found in the vertebrate and involve communication between neurons and smooth muscle cells by catecholamines and peptides. In one case the membrane events have been characterized (as an alteration in voltage-dependent pacemaker conductance). We have called this type of intercellular signalling "neurohormonal communication" because it is clearly different from conventional synaptic transmission (Barker and Smith 1977). Whether such a form of communication occurs in the vertebrate central nervous system still remains to be established. Certain forms of pharmacologically-induced behavior suggest that it may occur as, for example, drinking behavior induced by intracerebral application of angiotensin (Fitzsimons 1971). [See Toates: "Homeostasis and Drinking" *BBS* 2(1) 1979.]

The observations schematized in I-K are examples of pharmacological actions of endogenous substances, which are unlike those of conventional neurotransmitters. The "modulation" shown in I involves alteration in postsynaptic neurotransmitter receptor-coupled conductance by various peptides. The effect in J is peptide alteration in voltage-dependent Ca^{++} conductance. The latter is thought to be a pharmacological correlate of the events described in G. The actions shown in I and J have not yet received physiological definition.

From these observations it seems clear that intercellular communication in the nervous system is unlikely to be limited to synaptic transmission mediated by neurotransmitters. The functionally distinct signals described thus far allow for considerable flexibility and subtlety in the communication of changes in excitability among neurons. The complexity of vertebrate and invertebrate behavior would appear to require complex patterns of excitability predicated upon multiple modes of intercellular communication. Even if we do not agree on what to label certain signals, it is time to recognize that they exist and need further study to understand their role in neuronal function. It would seem reasonable that, during this early stage of research on the subject, we dispel preconceived notions and remain open-minded, and that by making accurate observations we will be able to group signals into various categories based both on the anatomy of the cells involved and the functional and physiological consequences of the signal communicated.

by **Alan A. Boulton**

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The trace amines: neurohumors (cytosolic, pre- and/or postsynaptic, secondary, indirect)? The target article by Dr. Dismukes is timely and pertinent. His definitions – four in all – seem to me, however, to be inadequate. As his review of the relevant literature is more than adequate and his interpretations similar in many respects to what I would myself wish to make, I will limit my comments to a description of the potential synaptic function of the trace amines. This is desirable in its own right, but more especially since Dismukes appears to have interpreted my earlier hypothesis rather more narrowly than was intended. I did not wish to imply in that paper that the trace amines were released along with an existing transmitter (i.e. the concepts of co- or false transmitters), nor were they envisaged as evincing classical postsynaptic effects, although of course they might do either or both of these things. Rather, I tried to describe possible synaptic interactions that have been referred to as neuromodulatory or neuroregulatory.

In more recent reviews than the one quoted by Dismukes, I have suggested (Boulton 1976a; 1978; 1979) that . . . "their mechanism of action may be that of 'synaptic activation,' acting either directly or indirectly in the process of neurotransmission. . . . It is envisaged that one or more of the trace amines, synthesised within the cytoplasm of the neurone, interacts with synaptic membranes in such a way as to cause a continuous activation. This activation would be sufficient to create miniature synaptic potentials, separated spatially and temporally, similar to those produced from the release of packets (quanta) of conventional transmitter substances which depolarize (or hyperpolarize) the membrane in a fashion similar to that described by Katz (1969) with respect to peripheral junctions. This would represent the 'direct' mode of action of the trace amines (see Fig. 1). Alternatively, this phenomenon could arise in an 'indirect' manner as a consequence of the more classical amines being released from their storage sites by the trace amines, a property they are known to possess. It is conceivable that such a phenomenon might be a requirement (i.e. to maintain the synaptic area in a 'state of readiness') for synaptic transmission when transmitter substances are released in amounts sufficiently large to permit the postsynaptic effects of the impulse."

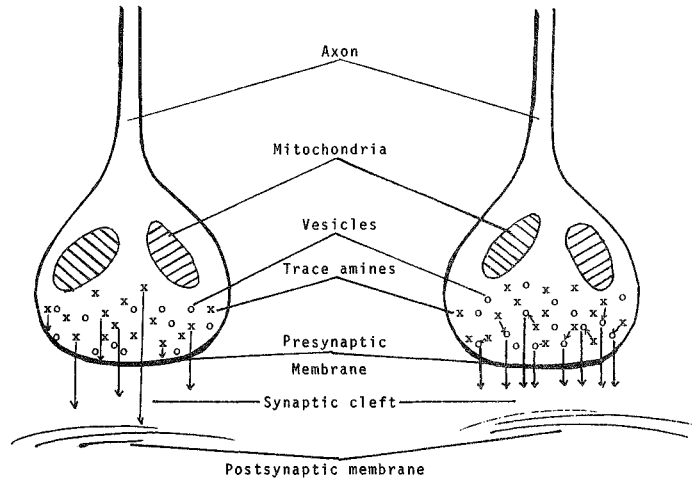

Figure 1 (Boulton). Possible mechanism of action of the trace amines.

It was of course also mentioned that some of the trace amines might, in discrete locations, act as neurotransmitters in the conventional manner.

Using the terminology advanced by Dismukes – if I understand him correctly – one could describe the possible conventional transmitter actions of the trace amines simply as synaptic neurotransmitters. It is not clear, however, how one would describe the other postulated actions of the trace amines without expanding Dismukes's definitions in some way. Perhaps without destroying the directness or appealing simplicity of his thesis, it is possible to expand or subcategorise the definitions. For example:

Neurohumor - source, site, type, mode; where source = neuronal, glial, cytosol, granules, etc.

site (of action) = presynaptic, postsynaptic, blood vessel, muscle, etc.

type (of action) = synaptic, nonsynaptic, etc.

mode (of action) = primary (similar to acetylcholine in peripheral junctions eliciting propagation of the impulse), secondary (eliciting subthreshold response), direct (action caused by the transmitter itself), indirect (actions mediated by a different substance), etc.

Using such a categorisation, it would be possible to describe the direct synaptic activation properties of the trace amines as:

"Neurohumor - cytosolic, pre- and/or postsynaptic, secondary, direct."

Similarly, the indirect action would become:

"Neurohumor - cytosolic, pre- and/or postsynaptic, secondary, indirect."

Terms similar to these would permit a description, at least in part, of the known or suspected properties of neurotransmitters, neuromodulators, neuroregulators, first or second messengers, and the like.

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by **W. Dale Branton *and* Earl Mayeri**
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Nonsynaptic interactions in* Aplysia *and their relation to vertebrate systems. We are studying communication between neuroendocrine bag cells and nearby target neurons in the abdominal ganglion of *Aplysia*. When Mayeri and Simon (1975) reported the first evidence for these interactions, it was suggested that they occur in the absence of synaptic connections, and the term "neurohormone" was used to describe the mediator. Further studies suggest that at least one of the responses (in target cell R15) is mediated by a peptide, egg laying hormone (ELH), and that ELH acts locally in a process that is intermediate between classical synaptic and blood-borne hormonal actions (Branton, Arch, Smock, and Mayeri 1978a; Branton, Mayeri, Brownell, and Simon 1978b).

We are in general agreement with the approach proposed by Dismukes: ELH fits several authors' definitions of a neuromodulator, but it is simpler to call it either a nonsynaptic transmitter or a neurohormone. We also agree that it would be unwise to restrict the term neurohormone to substances producing unconventional, voltage-dependent conductance changes, since some target cell responses in the abdominal ganglion, except for their prolonged time course, are quite similar to conventional synaptic responses (Brownell and Mayeri 1979).

It is useful to compare the properties of the bag cell system (see Mayeri *et al.* 1979a; b) to those proposed for the locus coeruleus noradrenergic system. In both systems: 1) release is nonsynaptic, 2) axons are profusely branched and there are large numbers of target neurons, and 3) responses of target neurons have slow onsets and prolonged durations. In both systems these combined features serve to regulate large populations of neurons over prolonged periods of time, but there are differences in the details of operation which may reflect differences in function. The impulse activity in the noradrenergic system is slow and tonic and may serve to regulate behavioral states such as arousal by affecting target neurons in a slowly graded manner. In contrast, bag cell activity is episodic and is followed by a stereotyped behavior pattern associated with egglaying. Correspondingly, and episode of bag cell activity results in a stereotyped set of electrical events lasting for several hours in various target neurons. Some neurons involved in sensory, motor, or homeostatic functions are turned on, others are turned off, and transmission along the synaptic pathways in the ganglion is altered.

In addition to their similarities to the central aminergic systems, the bag cells may also be similar to several of the peptide-containing neuronal systems recently discovered in the CNS of mammals (see Elde and Hökfelt 1979). These systems, like the bag cells (see Mayeri *et al.* 1979a; and Haskins *et al.* 1979), often have profusely branched axons with varicosities spaced along them, and for substance P containing neurons in spinal cord there is electron microscopic evidence that these varicosities are nonsynaptic release sites (Barber *et al.* 1979). Although there is as yet no direct evidence for nonsynaptic release in the vertebrate peptidergic systems, the possibility of nonsynaptic transmission with functional properties similar to the bag cell system deserves consideration.

by **D. A. Brown**
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Neuromodulators. Dr. Dismukes puts into words a number of thoughts about neurotransmission processes which, I think, are already included (even if subconsciously) in the current conceptual repertoire of most neurobiologists. It has been clear for some time that the punctate and temporally-brief types of transmission process so intensively studied, such as those of muscle fibers and motoneurons, are unlikely to represent the sum total of possibilities within the central neurons system.

Indeed, for examples of "neuromodulation" we do not have to go as far as the invertebrate nervous system. What about the heart? Is not the control of voltage-dependent ionic currents in cardiac muscle fibers by epinephrine (Hauswirth et al. 1968) simply an instance of a "neuromodulatory" action of a transmitter? Dismukes raises the analogy of the sympathetic ganglion – rightly, in my mind. We might also go further here, for the experiments of Kuba and Koketsu (1974) show that the inactivation of K^+ currents in frog sympathetic ganglion cells – referred to by Dismukes – is essentially a "voltage-dependent" phenomenon, and hence more suited to the modulation of ongoing discharge than for the direct initiation of activity.

The sympathetic ganglion illustrates an additional point about "Dale's principle" – namely, that *the same* transmitter (acetylcholine) may produce two (or three?) different effects on the *same* postjunctional cell; if one transmitter can do this, there's hardly much need for more than one to be released! It also seems most unlikely that the muscarinic "modulating" effects of acetylcholine released in the ganglion are triggered at the same subsynaptic focus as the fast nicotinic effects – a further example of transmitters acting remotely. On intracellular second messengers, one can hardly add more at this stage, except that a) they are almost certainly necessary if the effect of a transmitter is to reduce a resting conductance, in order to amplify the effect; and b) the search should go beyond cyclic nucleotides.

Finally, on "remote" transmitter release: Dismukes considers release from varicosities, in which transmitter is stored in vesicles. But what about nonvesicular release? There are membrane carriers for most transmitters, and most carriers are bidirectional, and may be voltage-sensitive; so, if speed is not essential, carrier-mediated "modulator" release must be considered. The problem is to demonstrate or negate it experimentally.

by **Theodore H. Bullock**
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Communication among neurons includes new permutations of molecular, electrical, and mechanical factors. The target article by R. K. Dismukes does significant service. It concentrates upon the direct forms of molecular communication. However, it is worthwhile to note that additional degrees of freedom and opportunities for lability inhere in the permutations of these molecular mechanisms with electrical and with mechanical-geometric changes.

Some of these are well demonstrated; others are only very likely. Spira et al. (1976) have shown the modulation of electrical transmission by chemical transmission. Electrical field effects, such as occur in the axon cap region of the Mauthner neurons (Faber and Korn 1978), seem likely to be influenced by chemical modulation. The tuning of neurons – that is, their preference for some inputs, exemplified by the frequencey tuning curves of electroreceptors (Viancour 1978; 1979; Hopkins 1976; Bastian 1976; Scheich and Bullock 1974) – is most probably altered by Ca^{++} and other specific components of the milieu.

Reciprocally, the release, distribution, or influence of neurohumors – Dismukes's sense, including the other categories of transmitters and modulators – may plausibly be under the influence not only of the classical postsynaptic potentials but of the variety of more recently appreciated forms of membrane response such as long-lasting hyperpolarizations (Bloom et al. 1972; Weight 1974b; Libet 1970) and plateau potentials (Russell and Hartline 1978). Dismukes refers to some of these, but only as complex forms of response to neurotransmitters. They are also likely to be factors in the release, distribution, or effectiveness, insofar as much membrane potentials occur in dendrites that include presynaptic sites, and in axon terminals.

Another likely source of influence upon neuronal communication is the movement of processes, the change in geometry with otogeny, adequate stimulation, excessive stimulation, or response – among other conditions that might induce mechanical changes in contacts or proximity. This is the more likely on newer views of ample and locally varying extracellular space in the CNS (Cragg 1979).

Dismukes's useful emphasis upon the variety of molecular communication should remind us of the additional variety of forms and permutations involving electrical and geometric factors.

***by* Larry L. Butcher**

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What's in a name? A neuromodulator by any other name would function just as well. The recent explosion in the use of correlative histochemical and electrophysiologic methods to elucidate the functional significance of various chemicals thought to be important for information flow in the nervous system has created not only a wealth of new information concerning the ways in which neurons communicate with one another and with other tissue elements, but it has also generated data that urge re-examination of such time-honored formulations as the one-neuron, one-transmitter doctrine attributed to Sir Henry Dale (Butcher and Talbot 1978; Talbot 1978). Dismukes has highlighted some of these developments and their theoretical implications.

Substances involved in neuronal communciation processes can be analyzed along at least two dimensions (Butcher and Talbot 1978): 1) the distance between the locus of release and the site of action of the neurochemical (i.e., length of communication channel), and 2) the duration of its action. The term "neurotransmitter" has been reserved for naturally occurring chemicals with relatively short-acting effects at sites relatively close to the locus of release. By comparison, "neurohormone" has been applied to neurochemicals with relatively long communication channels and durations of action. In general, substances traditionally conceived as having a neurotransmitter role are smaller molecules than neurohormones, and this feature may be significant. Substances operating over longer distances may possess greater structural complexity to preserve specificity of signal transmission, since the probability of noise introduction (e.g., increase in the number of different physiologically significant chemicals in the circulatory system) increases as a function of communication channel length.

This broad distinction between neurotransmitter and neurohormone appears to be accepted by Dismukes as meaningful. There exists a "gray area" in the continuum encompassing the categories of neurotransmitter and neurohormone, however, one into which an increasing number of substances appear to be falling. Among these chemical compounds are prostaglandins and various neuropeptides (e.g., Substance P, enkephalins) that have longer durations of action, different chemical structures, or different sites of action than those traditionally associated with neurotransmitters. In addition, substances for which a classic neurotransmitter role has been ascribed at certain places in the nervous system, such as acetylcholine and norepinephrine (see Butcher and Talbot 1978), may fall into this gray area at other neural loci. In this regard it is important to realize that the function of a given neurochemical cannot be divorced from the particular temporal and spatial matrix in which it is located. Because a certain endogenous chemical compound subserves a certain function at one locus does not necessarily mean that it subserves that same function at all loci where it is found.

What, if anything, do we call substances that do not appear to fit primarily into the category of neurotransmitter or neurohormone? Dismukes rightly questions the indiscriminate labelling of such chemical compounds as neuromodulators, neuromediators, or neuroregulators – terms that, according to Dismukes, do not appear to have been rigorously defined or applied by most neuroscientists using them. To be useful, a designation like neuromodulator must apply to substances operating in functional capacities different from those of, for example, neurotransmitters or neurohormones; there is no utility in having different terms for the same function of different chemical compounds.

Since knowledge about neurochemicals in the gray area is only now accumulating, Dismukes may be right in suggesting that perhaps it is too early to rigidly characterize various neuronal communication processes. Within this vast gray region of ignorance, however, we can ask whether or not there is any experimental evidence to suggest that concepts other than neurotransmitter or neurohormone might be at least heuristically useful at the present time. An affirmative answer to this question depends in large part on how we define neurotransmitter and neurohormone. Dismukes defines a neurohumor as "...any substance released by a neuron to alter the activity of other cells, adjacent or distant." A synaptic neurotransmitter is a ". . . neurohumor which is released for diffusion to an adjacent excitable cell (and to autoreceptors, when present)." But autoreceptors are apparently located on the same neurons from which the transmitter is released, and this morphologic condition appears to violate the definition of neurohumor proposed by Dismukes – namely, a substance that modifies the activity of *other* cells. The possibilities of nonsynaptic neurotransmission, as well as the release of monoamines into the cerebrospinal fluid, remain intriguing, but, as pointed out by Dismukes, such neuronal communicative processes are as yet demonstrated.

My current conception of a neurotransmitter or neurohormone involves a notion similar to Dismukes's general term "neurohumor." Such substances are endogenous compounds released from neurons at physiologic levels of excitation that transverse communication channels of variable length to interact with target structures to directly initiate the sequence of events leading to activity in those target areas. The critical feature here is that the neurohumor signal conveys information from one tissue element, a neuron, to one or more tissue elements other than those from which the transmitter was released. How this information is treated at the receiver to produce activity depends on the nature of the decoding mechanisms of the receptors or other physiologic entities of the target structures.

Neurochemicals that modify the activity of the same neuron from which they are released are not considered to be neurohumors, as defined by Dismukes. Similarly, substances that do not initiate the sequence of events leading to changes in the activity of target structures, yet alter subsequent or simultaneous neurohumor effects, are not judged to be neurohumors. Is there any evidence for the existence of such chemical compounds? Presently available data suggest that there is. In a cogent analysis Starke et al. (1977) have reviewed several papers that demonstrate the existence of angiotensin, prostaglandin, muscarinic, α-adrenergic, β-adrenergic, opiate, and dopamine receptors on presynaptic adrenergic neurons. The function of neurochemicals acting on these presynaptic receptors appears to be to modify the release, and hence the effect, of catecholamines on postsynaptic target structures, rather than to initiate propagated antidromic activity in the afferent neurons. When the transmitter acts upon the same neuron from which it is liberated to alter subsequent transmitter release, then the locus of that interaction has been called an autoreceptor.

In a similar vein, Sjöstrand and Swedin (1968) have shown that the motor response of the isolated vas deferens to hypogastric nerve stimulation could be potentiated by adrenaline, noradrenaline, acetylcholine, histamine, serotonin, angiotensin, bradykinin, and Substance P at doses 10 to 1000 times lower than those required for these substances to produce direct contractions of the vas deferens. Sjöstrand and Swedin suggest that the most likely site of action of these potentiating substances is postsynaptic at the smooth muscle. No such effect was produced by oxytocin or vasopressin, but prostaglandin E_1 and F_1 effected inconsistent responses.

Since, in the context considered, these compounds, do not appear to fit the definitions of neurotransmitter or neurohormone as conceived by Dismukes, might it not be justifiable, if only for heuristic reasons, to label such substances neuromodulators, with the category of agents considered by Starke et al. (1977) being presynaptic neuromodulators, and those investigated by Sjöstrand and Swedin (1968) being postsynaptic neuromodulators?

***by* Douglas L. Chute**

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Do new concepts of molecular communication rejuvenate old concepts of behavioural "states" in learning and memory? Dismukes concludes that nonsynaptic transmission (if it occurs *in vivo*) may provide a model for state regulation in the organism. This could rejuvenate state or field theories in psychology, which have had a mixed degree of respectability since Lashley. Basically, these theories have eschewed a strict connectionistic or cellular approach to learning and memory, based on specific pathways or predetermined connections; instead they have concentrated on more global interactions of

large cell assemblies, neuronal environment, and macro-electrical field effects (e.g. John 1972). A variety of nonsynaptic transmitters and neurohormones (to use Dismukes's potentially helpful nomenclature) have been shown to affect learning and memory (e.g. de Wied, Bohus, van Ree, and Urban 1978; Flood, Vidal, Bennett, Orme, Vasquez, and Jarvik 1978). Although the effect of neurohormones may be specific in terms of known or suspected receptors in CNS tissue (e.g. for ACTH: Watson, Richard, and Barchas 1978), they could be interpreted as functioning in a manner consistent with field theories – that is, relatively independently of specific connections. A number of neuroscientists (e.g. Bullock 1974) have argued against any field theories. Cooper, Bloom and Roth (1978) claim it is "nearly impossilbe to subject the theory to experimental testings." Even if true, the speculations in Dismukes's conclusions have little value. Clearly, the psychologist interested in behavioural states or field theory must rely on the reality of nonsynaptic transmission, CSF transport of serotonin, peptides, (etc.), and neurohormone modulation both to supply an experimental avenue for empirical testing and as a physiological substrate for theoretical modeling.

I suspect that the evidence Dismukes musters from nonsynaptic transmission may not be as tidy as it seems and may not then justify the revitalization of the notion of behavioural state. Most of the evidence cited deals with catecholaminergic and serotonergic neurons, which, compared to GABA neurons, for example, represent a relatively small (though not inconsequential) part of the CNS. The evidence for nonsynaptic transmission represents an even more restricted population, potentially of a highly localized nature. These restrictions admittedly exist because work is presently limited technologically to neurotransmitters that have reasonably clear and unique synthetic pathways. Other evidence Dismukes cites would also seem to be restricted in the number of cells involved, or else be highly speculative – for example, bidirectional graded conduction, and neurotransmission effects caused by transmitter release from parts of the neuron other than the varicosities. I expect that other commentators will deal with purported fact from which the degree of speculation can be deduced.

The issues surrounding nomenclature are considerably more important than merely tidying up, or deciding how wide a synapse is. The inability of neuroscientists to agree on a definition of a modulator is at least as great an impediment as the psychologist's inability to define attention, arousal, state, or memory. Dismukes runs no mean risk, therefore, in his conclusion when he hopes for heuristic value in the concept of "modulation of functional state." Although I may have subscribed to a notion of state effects on memory (e.g. Chute and Wright 1973), there are many psychologists who might believe this to be a "pharmacological curiosity," and that Dismukes has found a physiological substrate as an "answer" where unfortunately there is no "question." Dismukes correctly points out the potential importance to animal behaviour and psychiatric disorders of work on transmitters and peptides. However, seldom does any substantive use seem to be made of the concept of state. Discussing the role of catecholamines in health and disease, for example, Laverty (1978) apparently never feels the need to invoke states, let alone nonsynaptic transmission.

This review of modes of neural integration and communication in conjunction with the commentaries will, I suspect, serve a useful purpose. Our own work on memory facilitation and cAMP, for instance, is difficult to interpret in a classical neurophysiological framework (Villiger and Chute 1979). One wonders why it should be possible to facilitate memory at all, unless there is deficit elsewhere. A great deal of biological investment in the development of central nervous systems presumably "costs" more to produce than comparatively small changes in second messengers, phosphodiesterase activity, peptides, or other neurohormones. How are state and specificity related?

Cooper et al. (1978) notwithstanding, I think new concepts of molecular communication have again raised the issues surrounding the behavioural states and field theories; presumably they supply a means of empirical evaluation for a variety of hypotheses and conjectures about the possible psychological effects of nonsynaptic transmitters and neurohormones.

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No real alternative to existing definitions of neuronal communication. I have read and re-read many times this article by Dismukes. With each succesive reading I experienced intensifying ambivalence. Positive, on the one hand, as I began to comprehend better some of the complex ideas with which the author was attempting to deal. For this I thank the author. Negative, on the other hand, as I sensed the very core issues somehow eluding Dismukes as he perhaps became caught up in the semantics of the imperfect language system upon which we all depend for our own communication. This may in turn be responsible for my feeling that Dismukes could have performed a greater service by having been more critical of that literature which apparently provided the impetus of this paper. Even more important, though, is the apparent lack of any concrete discussion of structure-function relations which should represent the major goal in this particular journal.

First, my concerns about literature assessment: One main body of evidence upon which Dismukes builds his case for re-evaluation of neural communication modes pertains to recent studies in which substantial numbers of monoamine neuronal elements are shown to approximate other cellular elements which are non-neuronal (e.g. Descarries, Beaudet, and Watkins 1975). While careful to point out that there is less than complete agreement about the ubiquity of this phenomenon, Dismukes fails to provide the critique of methodological factors so germane to valid identification of the very monoamine neurons he chooses to discuss (see Zaborszky, Leranth, and Palkovits 1979 for analysis of this problem). Most neuroscientists will agree that our present comprehension of CNS mechanisms is at best rudimentary. Accepting this, a major responsibility in neuroscience fields must be to characterize fully the strengths and limitations of techniques upon which conclusions are based. Little in Dismukes's paper demonstrates to me a careful consideration of either the reliability or the validity of these methods in terms of his major thesis. As such, the potential impact of his attempted conceptual development appears weakened.

My primary objection to Dismukes's approach is his blatant (if not deft) avoidance of describing meaningful structure-function relationships (i.e. brain-physiology, brain-behavior). To me, statements such as ". . . co-ordinating or tuning the activities of large arrays of target neurons." or ". . . regulating the state of behavioral responsiveness . . .." are nondescriptive phraseology. The main issue has been missed. Exactly *what* does "tuning" or "regulating" connotate ("modulation"?)? The main issue of what "information" may be transduced in this "new" model is not addressed. Ultimately, I am left with the feeling that the broad scope of putative function subserved by these neurons may be no more clearly described than the general "autonomic tone" concept originally espoused by Brodie and Shore (1957).

In the end, this article boils down to a semantic debate about "transmission" versus "modulation," with the author rejecting any real distinction between the two. While I agree that the boundaries between these two concepts are less than clear, I cannot agree that blurring them further can assist neuroscientists in better operationalizing the main issues surrounding any future understanding of neuronal communication and its functional significance. At this early stage of scientific inquiry into CNS function, the real problem is not an inabiltiy to define terms – it is to understand the true meaning of the tools with which we study neural mechanisms, and to test working hypotheses, however imperfect, with these meagre approaches. In this respect I do not believe that Dismukes has succeeded in providing any real alternative to existing operational definitions of neuronal communication.

by **J. J. Dreifuss *and* M. C. Harris**

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Hypothalamic neurohumors as neurohormones and neurotransmitters. In his article Dr. Dismukes suggests that our concepts of intercellular communication in the nervous system are in need of revision because of the discovery of new modes of action exerted on

neurones by biologically active substances. The author rightly challenges the value of using such ill-defined terms as "neuromodulator" or "neuroregulator" when referring to the agents responsible for unconventional modes of neural communication. Instead, he recommends a "flexible generic approach for substances released from neurones." The suggestion is to use *neurohumors* as an all-encompassing term for substances released into the extracellular space by neurones. Neurohumors acting on nonneuronal cells are designated *neurohormones*, and those acting on neurones, *neurotransmitters*. The latter may be synaptic or nonsynaptic transmitters, depending on whether they act on an immediately adjacent neurone or on a group of neurones at some distance from the site of release.

Though, on the surface, this proposal may appear useful, closer examination shows a number of ambiguities, and we feel Dismukes is really replacing one set of confusing definitions by another set that is equally open to criticism. The confusion promulgated by his proposed definitions is well illustrated by reference to the hypothalamic neuropeptides.

In recent years it has become increasingly evident that the hypothalamic hypophysiotrophic and neurohypophysial hormones, which are synthesized within and liberated from endocrine neurones in ways essentially similar to those of nonendocrine neurones (Scharrer 1969), must also be considered to be neurotransmitters as defined by Dismukes. For, although they are liberated into the circulation, they have also been localized in axon terminals throughout the central nervous system.

This dichotomy of function is well illustrated by the neurohypophysial hormones. For nearly thirty years it has been known that the vasopressin- and oxytocin-producing neurones of the hypothalamus project not only to the posterior pituitary, but also to extrahypothalamic regions of the brain. Gomori-positive neurosecretory axones reach several areas of the central nervous system and contact or even surround perikarya in these regions (Barry 1954). With the recent availability of specific antisera and their application to immunocytology and radioimmunoassay, these early findings have been substantiated. Thus vasopressinergic and oxytocinergic pathways originating in the magnocellular hypothalamic nuclei have been traced to the septum, the amygdala, the hippocampus, the hindbrain, and even to the spinal cord (for example, Buijs 1978). Moreover, when applied by iontophoresis to mammalian neurones, vasopressin has actions akin to those of a neurotransmitter (Nicoll and Barker 1971). There is also good evidence for a central role of vasopressin in more complex functions, such as thermoregulation (Kasting et al. 1979) and memory (de Wied 1976). Furthermore, vasopressin is present in much higher concentrations in cerebrospinal fluid than in plasma, and the possibility must therefore be considered that it may reach some targets by this route (Dogterom et al. 1978).

Whilst discussing the various means by which vasopressin may be liberated, it is probably worthwhile to point out that, although neurohypophysial axons are renowned for the occurrence of varicosities, there is no evidence whatsoever that these make any form of synaptic contact.

Vasopressin is only one example of the diverse forms of liberation and sites of action of hypothalamic peptides. For example, Thyroid-Stimulating-Hormone-Releasing-Hormone has been used an an antidepressant, and Luteinizing-Hormone-Releasing-Hormone released into the hypophysial portal blood is known to affect the discharge of hypothalamic neurones and is even released from autonomic axones (Jan et al. 1979).

From these few examples it is clearly difficult to place all neuroactive substances into the somewhat restrictive categories suggested by Dismukes. Such an exercise may even be harmful, since, when a substance has been categorized, the temptation is to ignore actions that do not fit that category. Consequently, whilst agreeing that concepts of intercellular communication should be revised, we would suggest that for the moment, since circumspections outnumber facts, the problem should be left as open as possible. In the meantime, the terms proposed by Dismukes should perhaps be used to describe *functions* rather than substances. This would overcome the difficulties of classifying single substances with multiple functions.

***by* Adrian J. Dunn**

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Molecular signals released by neurons. Dismukes has done an excellent service to the neuroscience community by raising, in print, issues that have hitherto been discussed only informally. Regardless of the outcome of the controversy surrounding the percentage of terminals of central noradrenergic, dopaminergic, and serotonergic neurons with postsynaptic specializations, it is clear that molecules released by neurons may directly interact with cells that are not adjacent to the releasing cell, so that our traditional concepts of intercellular communication must be revised. That several authors have chosen to address the issue of classification of "neurocommunicators" indicates concern that the literature is confused (and will become more so) by the lack of rigid definitions of terms such as "neurotransmitter" or "neuromodulator."

The first semantic issue that needs clarification is whether the prefix *neuro-* implies the releasing cell or the affected cell. Current usage favors the former; neurohumors, neurotransmitters, and neurohormones are considered to be released by neurons. Unfortunately, neuroregulators and neuromodulators could well be construed to be regulators or modulators of neurons, and Barchas et al. (1978) established criteria for neuromodulators on this basis. Nevertheless, it may be difficult to persuade neuroscientists to refer to acetylcholine in autonomic ganglia as a neurotransmitter, but at the neuromuscular junction as a "myoregulator" or "myotransmitter."

Attempts such as those of Barker (1978) or Dismukes to classify neurohumors try to combine aspects from a number of modalities. Regrettably, I have not seen Bloom's (to be published, 1979) attempt to deal with this; it appears to be of heuristic value but may not aid classification now. I agree with Dismukes that the term neuromodulator, which is now used so ambiguously, should be discarded. Barker's definition is too rigid, it conflicts with current usage by others, and it is already apparent that the distinction between a neurotransmitter and a modulator (by his definitions) may not always be clear. Dismukes's classification is primarily based on the distance between transmitting and receiving cells. It is confused by the introduction of factors relating to the modality of the effect produced on the target cell, and by blurring of the definitions so that there is overlap between nonsynaptic transmitters and neurohormones. Also, trophic substances released by neurons onto adjacent cells must be classified as neurotransmitters, yet their role is more consistent with that of classical hormones.

I propose the following definitions, which are consistent with much current usage. These definitions refer to functions and not the molecules, which may classify differently in different locations. Target cells need not be neurons. *Neurohumor: Any substance released by a neuron that alters the activity of other cells, adjacent or not.* This is Dismukes's definition. *Neurotransmitter: A neurohumor that effects changes of membrane potential in target cells.* Presently known neurotransmitters act on adjacent or nearby cells. If desired, the neurotransmitter category could be subdivided into synaptic and nonsynaptic, but present techniques do not permit empirical distinction. *Neurohormone: A neurohumor that alters the activity of target cells other than by altering their membrane potentials.*

These definitions dispense with the criterion of distance used in Dismukes's classification, and they substitute the criterion of membrane potential changes. Barker's "neuromodulators" would be classified as neurotransmitters because they effectively alter the postsynaptic membrane potential, albeit by modifying the effectiveness of other neurotransmitter inputs. Neurohormones would include classical hormones such as neurohypophyseal oxytocin and vasopressin, as well as trophic factors released by neurons. Transport may occur in channels such as blood vessels or the cerebral ventricular system (Dunn 1978), but this is not essential to the definition (many definitions of hormones do not require this). The definitions are not mutually exclusive, and it is conceivable that a neurohumor in a particular location could serve both as a neurotransmitter and a neurohormone.

I must add a comment on the frequently assailed Dale's Principle. Although it has been known for many years that individual neurons

release more than one chemical substance, many critics seem obsessed with the co-existence of alleged function of more than one neurotransmitter in a particular cell. While the letter of Dale's Principle may be violated, surely an important aspect of the Principle is that all terminals of a single neuron should function similarly. Even if more than one neurotransmitter were to be released by a given neuron, all terminals would release the same neurotransmitters. Neuroscience is not yet ready for a neuron to release different neurotransmitters at different terminals.

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by **Glen R. Elliott *and* Jack D. Barchas**

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Neuroregulators: neurotransmitters and neuromodulators. Although the concept of chemical transmission between some neurons was firmly established in the 1920's, it was not accepted as the principle means of neuronal communication in the central nervous system until thirty years later. Since that time, tremendous technical strides have expanded greatly the ability of scientists to manipulate and monitor brain cells electrically and chemically. Much of the resulting wealth of information has served to confirm that certain substances do mediate transmission of electrical impulses between nerve cells. However, the data have also necessitated refinements of the initially simplistic model. Thus, a variety of regulatory mechanisms of synaptic activity has been proposed, such as direct and indirect feedback loops, pre- and postsynaptic receptors, and specific inactivating mechanisms. Still, these additions have not altered the basic concept that neurons communicate as follows: an elecrical impulse travels down an axon until it reaches the synapse, a specialized modification of both neuronal membranes; presynaptic structures provide for the synthesis and storage of the chemical transmitter, a portion of which is released into the synaptic cleft by the electrical impulse; the transmitter then diffuses across the cleft to interact with specific post-synaptic membrane receptors to produce a change in the membrane potential, thus converting the message back into an electric signal.

In his excellent article Dr. Dismukes concentrates on many other recent findings that seem not to fit well into the above model. He points to electrophysiological and electron microscopic data suggesting that some "transmitters" are released nonsynaptically, and he cites evidence that some large neurotropic molecules may undergo retrograde transport from "postsynaptic" to "presynaptic" neurons. He also mentions reports of single nerve cells containing two or more neuroactive substances – an apparent violation of the long-accepted Dale's Principle, which postulates that a single neuron can secrete, at most, one transmitter. Finally, he describes a variety of complex electrophysiological responses of neurons that cannot be explained readily by simple synaptic mechanisms. Altogether, these observations powerfully argue the pressing need for broader perspectives from which to evaluate neuronal function.

In response to this need, Dismukes has proposed that the increasingly wide variety of neuronally-active compounds, which he defines generically as "neurohumors," be subdivided into three broad classes: synaptic (or junctional) neurotransmitters, which correspond to the classical neurotransmitters; nonsynaptic (or nonjunctional) neurotransmitters, which resemble synaptic neurotransmitters but have more distant or multiple targets; and neurohormones, which also affect multiple or distant target cells but lack specific membrane receptors and electrical responses that characterize the former two categories. Under this system, compounds are first defined by their origin – neurons – and then by their target – near or distant cells with or without specific receptors.

We agree with Dismukes that neurochemistry urgently needs a new, more encompassing nomenclature for categorizing the burgeoning mass of substances that appear to be important for neuronal function. Such a nomenclature must be narrow enough to exclude those compounds that patently have no specific effect on neuronal communication, yet broad enough to include as many appropriate compounds as possible, even if their specific functions remain unclear. Otherwise, it may introduce unwitting biases that can subtly but forcefully discourage potentially fruitful avenues of research. For example, studies of acetylcholine led investigators to conclude that metabolism was the principle means by which neurotransmitters were de-activated after release. It took the brilliant studies of Julius Axelrod to demonstrate the importance of re-uptake in the inactivation of norepinephrine – a de-activating mechanism that appears to have more general applicability than does metabolism.

We believe that a nomenclature focused on substances produced by and released from neurons – i.e. neurohumors – is too restrictive. Information already available suggests that such a restriction is inappropriately narrow. Thus, it would exclude adrenal glucocorticoids, compounds known to affect catecholamine synthesis and, thus, catecholamine activity. Similarly, it would exclude glucose, even though it has specific neuronal receptors in the hypothalamus that appear to be

Table 1. (Elliot & Barchas). *Criteria for establishing the identity of a neuroregulator in the central nervous system*

Neurotransmitter
The substance must be present in presynaptic elements of neuronal tissue, probably in an even distribution throughout the brain.
Precursors and synthetic enzymes must be present in the neuron, usually in close proximity to the site of action.
Stimulation of afferents should cause release of the substance in physiologically significant amounts.
Effects of direct application of the substance to the synapse should be identical to those produced by stimulating afferents.
Specific receptors that interact with the substance should be present in close proximity to presynaptic structures.
Interaction of the substance with its receptor should induce changes in postsynaptic membrane permeability leading to excitatory or inhibitory post-synaptic potentials.
Specific inactivating mechanisms should exist that stop interactions of the substance with its receptor in a physiologically reasonable timeframe.
The effects of stimulation of afferents or of direct application of the substance should be equally responsive to and similarly affected by interventions at postsynaptic sites or through inactivating mechanisms.
Neuromodulator
The substance cannot act as a neurotransmitter, as defined above.
The substance must be present in physiological fluids and have access to the modulatory site in physiologically significant concentrations.
Alterations in endogenous concentrartions of the substance should affect neuronal activity consistently and predictably.
Direct application of the substance should mimic the effect of increasing its endogenous concentrations.
The substance should have one or more specific sites of action through which it can alter neuronal activity.
Inactivating mechanisms should exist that account for the time course of neuronal effects induced by endogenous or exogenous changes in concentrations of the substance.
Interventions that alter the neuronal effects of increased endogenous concentrations of the substance should act identically when concentrations are increased by exogenous administration.

Modified from: Barchas, J. D.; Akil, H.; Elliott, G. R.; Holman, R. B.; and Watson, S. J. (1978) Biochemical neurochemistry: neuroregulators and behavioral states. *Science* 200:964–973.

central to regulation of hunger mechanisms. On the other hand, a nomenclature that subclassifies compounds only according to the physical distance and lack or presence of specific receptors seems too broad. For example, pituitary hormones are released from neurosecretory cells. Are they neurohumors even if they exert their effects on non-neuronal tissues?

Interestingly, most endogenous substances have been named either for their organ of origin or for their biological activity. Thus, in the early 1950's there was considerable debate about whether the the newly isolated compound 5-hydroxytryptamine should be named "enteramine," for the enterochromaffin cells from which it could be extracted, or "serotonin," for its effect on blood vessels. More recently, a similar controversy developed over the endogenous opioid peptides, centrally-active substances with morphine-like activity. The first two peptides were called "enkephalins," since they were isolated from the brain. However, a subsequent agreement was reached to call the entire class of these compounds "endorphins," to indicate their activity.

As described in an earlier paper (Barchus et al. 1978), we believe that focusing on function, rather than origin, provides a better system for classifying neuroactive compounds. We have proposed the generic term "neuroregulators" to define those substances that play a role in communication among neurons. Thus, they differ not only from compounds involved primarily in cell maintenance, such as oxygen and sugars, but also from "second messengers" like cyclic AMP and cyclic GMP, which translate neuroregulator signals into cellular events. Neurotransmitters, one of two major subcategories of neuroregulators, correspond both to the classically-defined neurotransmitter and to Dismukes's synaptic neurotransmitter. Neuromodulators, our other major subcategory, will undoubtedly be subdivided further as we better understand possible modulatory functions. One subtype might be neurohormones like the adrenal steroids, which are released from peripheral organs to provide long-term adjustments in the activity of some neurons. Other neuromodulators might act through neurotransmitters, interfering with receptors or with de-activating mechanisms to block or prolong transmission. Only now are techniques becoming available to explore such possibilities. Proposed criteria for identifying neuroregulators and for distinguishing neurotransmitters from neuromodulators are listed in Table 1.

Thus, we are in accord with Dismukes about the need for a new system for beginning to classify the many fascinating compounds that neuroscientists have discovered in the past twenty years. Just as neuronal communication is the focus of the neurosciences, so too should it be the center of this broader nomenclature. By concentrating on that process, it may be possible to begin to understand how the seemingly simple act of several thousand molecules traveling across a few Angstroms can be harnessed to produce the myriad complex tasks that we recognize as normal brain function.

by **Peter D. Evans**

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Modulatory actions of an identified octopaminergic neurone at the locust neuromuscular junction. One example of a nonsynaptically released biogenic amine with modulatory action, not discussed by Dismukes, is that of the phenolamine, octopamine, at the locust neuromuscular junction. This preparation presents a unique opportunity to study the modulatory actions of octopamine, since an identified octopaminergic neurone projects to the muscle concerned (Evans and O'Shea 1977; 1978; O'Shea and Evans 1979).

The extensor tibiae muscle of the hindleg of the locust *Schistocerca americana gregaria* is innervated by two excitatory motorneurones, one inhibitory motorneurone, and by the modulatory neurone. The latter is one of a group of dorsally-unpaired medial (DUM) neurosecretory cells and has been called DUMETi (Hoyle *et al.* 1974). The soma of the DUMETi neurone has been physiologically identified, isolated, and shown to contain octopamine (Evans and O'Shea 1977; 1978). The terminals of the DUMETi neurone on the muscle are reported not to form discrete synaptic structures but rather to be "blindly ending neurosecretory terminals" (Hoyle *et al.* 1974).

Electrical stimulation of DUMETi alone produces very little effect on tension in the extensor muscle, but it profoundly alters the actions of the slow motorneurone to this muscle (Evans and O'Shea 1977; O'Shea and Evans 1979). DUMETi potentiates the tension induced in the extensor muscle by the slow motorneurone, as well as increasing the size of its EJPs. The actions of DUMETi are of a prolonged time course and far outlast its period of stimulation. These effects can be mimicked by the application of low concentrations of octopamine to the muscle. It appears that octopamine can act both presynaptically and postsynaptically at the locust neuromuscular junction. At least some of the potentiation of tension is thought to be caused by presynaptic actions, and the effects of octopamine on increasing the rate of relaxation of the muscle must be postsynaptic.

There are many similarities between the actions of octopamine at the locust neuromuscular junction and the actions of biogenic amines at the vertebrate neuromuscular junction (Kuba 1970). It has been proposed that the DUMETi cell is part of an arousal system that serves to increase the responsiveness of the locust, possibly under stressful conditions, by placing a bias directly at the neuromuscular junction, and that this preparation can serve as a model for modulatory actions of octopamine in central nervous systems (O'Shea and Evans 1979).

In the marine mollusc *Aplysia* a parallel identified aminergic modulatory system exists. Here the 5-HT-containing metacerebral cells modulate the actions of certain buccal motorneurones both centrally and peripherally (Weiss et al. 1975; 1978). A dopaminergic modulatory system has recently been described in the gill of *Aplysia* (Swann et al. 1978). Neuromuscular transmission is also modulated by a variety of biogenic amines in the lobster, but here the skeletal muscles are not reported to receive a direct aminergic innervation (Kravitz et al. 1976).

Two specific comments on the Dismukes article are in order. First, in the studies of Descarries et al. (1977) the specificity of the uptake of ^{3}H-norepinephrine was claimed on the basis of blocking with desmethylimipramine. It should be noted that in the insect nervous system this compound is a potent inhibitor of a high-affinity uptake system for octopamine (Evans 1978a). It is not yet clear if specific octopaminergic neurones exist in the vertebrate CNS (see Evans 1978b), but if they should, then care will be needed in the interpretation of such electron microscope autoradiographic studies. Second, Dismukes quotes the work of Brownstein et al. (1974) as revealing the coexistence of serotonin, octopamine, and acetylcholine in the same identified neurones in *Aplysia*. It should also be noted that, at least as far as octopamine is concerned, McCaman and McCaman (1978) could not reproduce these results.

It would seem that invertebrate preparations offer many advantages for the study of aminergic modulation at the identified cell level, and that the results obtained will be of significance to modulatory systems in general.

by **Ernst Florey**

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Modulation of neuronal function – a not so new concept. Dismukes provides a timely review of a phenomenon that it has become fashionable to refer to as modulation. The aim of his paper is to provide "a systematic approach to nomenclature," and to serve as a springboard for a discussion of implications of nonsynaptic release of modulatory agents for neuronal information on processing.

The task is important, because the concepts under discussion signal a revolution in our thinking about the functioning of the nervous system. Dismukes is not altogether original in his approach; in fact, much of what he has to say has been said better by others, and many of his statements are far too general to be of use to the reader. The statement that "invertebrate neurons also possess gap junctions, which are thought to provide a means of synchronizing electrical activity" is of no more help than the observation that "neuropeptides produce diverse effects in invertebrates."

The author contrasts "excitation and inhibition, as conventionally defined" with "nonconventional actions" – modulation. He ignores the fact that neurophysiologists as well as neuropharmacologists have, for

some years now, learned to recognize several forms of transmitter action and to distinguish several types of "inhibition" and "excitation," for which they have sought – and are seeking – explanations in terms of the molecular (and ionic) mechanisms involved. There is no longer such a thing as "simple inhibition," or "simple excitation," as he puts it.

Such criticism should not detract from the main merit of Dismukes's paper, however: his effort to point out possibilities of neuronal communication that go far beyond the processes commonly associated with synaptic transmission.

His efforts towards formulating a definitive nomenclature are not as successful as one would wish. His definition of *neurohumor* as a generic term for any substance released by a neuron "to alter the activity of other cells" is far too broad to be useful – quite apart from the unjustified teleological implications. The definition equally applies to acetylcholine and to potassium – unless it is considered that the release of potassium is "accidental" and is not intended by the neuron "to alter the activity of other cells," even if such an effect occurs.

The same kind of unwarranted teleology is employed in the definition of *synaptic transmitter:* "a neurohumor which is released for diffusion or transport to multiple excitable cells." What if, together with the transmitter substance proper, the nerve terminal also releases a binding protein or an enzyme (as has recently been shown for noradrenergic terminals)? Does the terminal release them by accident, unintentionally? The omission of the action of the transmitter substance from the definition is dangerous because it renders the definition useless. And what are multiple cells?

Dismukes's definition of *neurohormone* states that it is "capable of regulating multiple distant target cells, which do not necessarily have membrane receptors or electrical responses." One must ask whether such cells have ever been found that have no membrane receptors or electrical responses. Presumably the implication is that the receptors interact with the neurohormone, and that the electrical responses might be the effect of the neurohormone. But how else would an excitable cell respond to a neurohormone but by a change recognizable as an "electrical response" (change in membrane properties that can be defined in electrophysiological terms)? This definition, too, requires revision.

In his discussion of "What is a Modulator?" Dismukes quotes my definition of modulator substance, published in 1967 (Florey 1967). This definition, incidentally, is also quoted in a recent review of synaptic modulation by Kupferman (1979). I wished my earlier (Florey 1960) discussion and definition were quoted instead, since it is more appropriate:

I then stated that a substance originating in nerve tissue "may normally act as a modulator of such processes as synaptic transmission, conduction of nerve impulses, or of cellular excitability." And I further said that "a transmitter substance must not necessarily act differently from a modulator substance. The chief distinction of a transmitter would be the dependence of the release on orthodromic nerve impulses occurring in the nerve cell which produces the compound, while the modulator substance would be released in a continuous or intermittent secretion process. Furthermore, we would assume that a transmitter substance is always released from nerve endings while a modulator substance may be released at any specialized or even unspecialized region of a cell. It might be produced by glia cells or the cells of nerve sheaths."

This was probably the first formulation of the modulator concept, and I think it is still very appropriate – also in the context of Dismukes's discussion.

Having arrived at the historical perspective, I must take issue with Dismukes's statement that the concept of the synapse was introduced by Sherrington in 1906; in actual fact, Sherrington already introduced the term synapse in 1897 in Foster's "Textbook of Physiology." How this came about was explained by Sherrington himself in a letter he wrote to John F. Fulton, who published it as a footnote on p. 52 of the 2nd Edition of his "Physiology of the Nervous System" (Oxford University Press, 1943). Sherrington's use of the term was by no means "prescient," as Dismukes puts it: the neuron doctrine (first formulated by Waldeyer in 1891) was already firmly established (although not universally accepted) by the end of the nineteenth century and was not, as Dismukes assumes, developed during the first half of our century.

If I have been critical of much of the detail of Dismukes's article, I must also say that I enjoyed reading it. He provides a perspective that has important implications for neurobiologists, and he calls attention to a point of view which is all too often ignored. As a springboard for discussion, Dismukes's review certainly succeeds.

by **Robert Freedman**

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Electrophysiology is not sufficient to determine neuromodulatory function. Dr. Dismukes's article comprehensively summarizes a wide range of experimental evidence from neuroanatomists and neurophysiologists, casting doubt on the universality of a model of central nervous system synaptic transmission that has been widely held since the 1960's. While it is tempting to preserve the older model of synaptic transmission and to label other forms of synaptic or nonsynaptic neurotransmission as "neuromodulatory," caution must be observed to avoid confusing as teleological label with a physiological property. As a physiological process, "neuromodulation" generally means that the neurohumor in question acts to change the effect of other synaptic or nonsynaptic inputs on the postsynaptic cell. The teleological implication of the word "neuromodulator" is that the process allows the activity of the neurohumor to influence the flow of information carried over the more classical pathways. Dismukes's proposed nomenclature avoids confusing the two meanings by eliminating the term or its equivalents, such as "neuroregulators." He also points out, in the conclusion of his article, that the simple model of classical neurotransmission as an "algebraic addition" at the postsynaptic neuron may not be correct; two different inputs may have a far more complex interaction.

Studies of the actions of norepinephrine (NE) and gamma amino butyric acid (GABA) in monkey cerebral (Foote et al. 1975) and rat cerebellar cortex (Freedman et al. 1977) demonstrate the problems involved in using the physiological effect of a transmitter to predict its functional role. Both GABA and NE, applied locally by microiontophoresis, inhibited spontaneous activity of either cerebral cortical neurons or cerebellar Purkinje neurons. When the cerebral cortical neurons were excited by tape recordings of monkey calls, both neurotransmitters inhibited these evoked excitations less profoundly than they inhibited spontaneous activity. Similarly, in the cerebellar cortex, both neurotransmitters inhibited the spontaneous discharge of the cerebellar Purkinje neuron. When the neuron was excited by stimulating either of two afferent excitatory pathways, the climbing fibers or the parallel fibers, these excitations were inhibited by either neurotransmitter less profoundly than spontaneous activity was inhibited. Thus, in both the cerebral and cerebellar cortices, either NE or GABA is able to "modulate" the responsiveness of neurons so that they respond to afferent excitation preferentially over spontaneous discharge. Although NE raises membrane resistance and GABA lowers membrane resistance, this difference seems to be reflected only in differences in the configuration of the complex spike evoked by stimulation of the climbing fibers.

The "modulatory" actions of NE and GABA, described above in electrophysiological terms, are not sufficient to define their functional role in "modulating" information processing in the cerebral or cerebellar cortex. Other information that is needed can be derived from three lines of inquiry: the neuroanatomy of the neurons using NE or GABA as a neurotransmitter, the activity of these various neurons during the animal's behavior, and the action of psychotropic drugs. The cerebellar cortex lends itself particularly to this sort of inquiry, because its structure and function are currently better understood than that of cerebral cortex.

If structure is the ultimate expresion of function, then the function of GABA in the cerebellar cortex is different from that of NE. Neurons that

use GABA as a neurotransmitter are all intrinsic to the cerebellar cortex. The basket and stellate cells are both Golgi Type I neurons, which receive parallel fiber synapses and synapse on nearby Purkinje neurons. Based primarily on neuroanatomical investigation, Eccles et al. (1967) proposed that these neurons are used to inhibit groups of Purkinje neurons surrounding other groups that are being excited. Eccles thought this spatial limitation on the extent of an evoked excitation would increase the precision with which cerebellar neurons could regulate movement. The role of the "modulatory" effect, which preferentially inhibits spontaneous over evoked activity, might then be to ready these silenced neurons for any further incoming impulse, which might signal a modification in the information just transmitted. Since the extent of innervation of a particular basket or stellate cell is generally less than twenty Purkinje neurons, and since inhibitions last only several hundred milliseconds, the "modulatory" effect would be useful only for very small and very quick corrections in movement. By contrast, the noradrenergic innervation is a diffuse, widespread, extrinsic innervation that is not as closely tied to other cerebellar neuronal elements. Noradrenergic fibers entering the cerebellum from the locus coeruleus branch widely, including major branches to other brain areas (Ungerstedt 1971). As Dismukes points out, such a structure suggests that this system might be designed to modulate activity in response to changes in behavioral state.

Studies of the locus coeruleus and the cerebellar cortex during the behavioral changes of the sleep and waking cycle provide evidence for such an hypothesis (Freedman 1977). During rapid eye movement (REM) sleep, for example, the locus coeruleus neurons that project to cerebellar cortex fire more slowly (Chu and Bloom 1974). Perhaps, because of this decrement in noradrenergic activity, cerebellar Purkinje neurons fire more rapidly (McCarley and Hobson 1972) and are less responsive to afferent input (Pellet et al. 1973). This "modulatory" effect, which is consistent with the electrophysiologic effects of NE noted above, may therefore be responsible for the suppression of phasic movement during REM. Lesions of the cerebellar cortex permit the expression of these movements, disrupting REM periods. This action of the noradrenergic innervation would seem to be a wholly different "modulatory" activity than that proposed for GABA.

The exploration of the mechanism of action of psychotropic drugs is another means of examining the functional significance of complex, electrophysiological effects and nonclassical synaptic structures. We have proposed, for example, that neuroleptic antipsychotic drugs, by blocking the modulatory effects of catecholamines, may produce the decrease in responsiveness to external stimuli, which has been correlated with their therapeutic effect (Freedman 1977).

In summary, Dismukes has summarized an important, growing area in neuroscience. The further investigation of the functional significance of synaptic transmission in both its classical and nonclassical forms requires the synthesis of data derived from many different neuroscientific disciplines.

by **Glenn I. Hatton**

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Neuronal communication: don't forget the glia! Dismukes has succeeded in focusing our attention upon the emergent concept that much of functional importance, beyond that which occurs via synaptic interactions, transpires between cells in nervous systems. He has listed, cited evidence for, and generally clarified a number of important ways in which nervous systems may (and probably do) act, in addition to point-to-point conduction, in order to enable them to handle the level of complexity that we observe in behavior. Certainly, synaptic relationships, with all of their vicissitudes, could not be expected to account for the richness in quality and tempo observed in, for example, the delay between summated stimulus and stored inputs on the one hand and behavioral output on the other, that we humans rationalize as "free will." In my view, Dismukes has taken a well-directed first step on what may prove to be a journey of a thousand miles. One strongly hopes that the kind of thinking about actual mechanisms that is displayed in this article will be adopted by investigators everywhere who deal with central neural events.

Another thing for which the author is to be commended is his attempt to offer a rationally based, internally consistent nomenclature for substances released from neurons. Historically, such attempts at standardization have met with limited success, but hope springs eternal. Perhaps the nomenclature proposed by Dismukes is broad and sensible enough to take hold.

One task standing before those of us who have thought about and researched this problem of nonsynaptic modulation of neural activity is to add our pet examples to the list started by Dismukes. Before adding mine, I would like to make two further comments on the substance of the target article. In dealing with the topic of Dale's Principle and the notion of one neuron: one neurohumor, the author rightly points out that we must not assume that a cell containing two (or more) putative neurohumors can and does release these as modulators. It seems appropriate to add to this admonition the further caution that we must not assume, without solid evidence, that the presence of more than one neurohumor even implies that the cell in which they are found is in fact capable of *manufacturing* both (or all) of them. The recently demonstrated wide distribution of the enkephalins within the mammalian CNS leads one to wonder if what we are seeing may indeed be uptake and/or sequestration of these pentapeptides by some cells, rather than multiple synthetic sites. This could of course hold for the monoamines and the peptides such as Substance P as well, though the mode of entry into cells may differ depending upon molecular size (e.g., active transport or pinocytosis). In any case, until it has been shown that more than one neurohumor in a cell is manufactured for transport by that cell, it is probably unwise to assume that the cell can liberate more than one transmitter.

My other comment is one that will lead into my pet examples of nonsynaptic modulation of neural activity. Dismukes has shown himself to be a bit traditional in giving short shrift to the role(s) of glial cells in neural functioning. Ignoring this ubiquitous cell type when considering the modes of action available to nervous systems has resulted in a predictable amount of ignorance about their possible functional significance.

We know that glial cells can take up (control?) extracellular K^+ and, perhaps in the case of astrocytes, transport excess amounts of the ion to the blood (see Orkand 1977 for review). Recent studies of the rat hypothalamo-neurohypophysial system have shown that a mild degree of dehydration, such as that produced by 4–12 hours of water deprivation, is accompanied by retraction of many of the five astrocytic glial processes that are normally interposed between the magnocellular neurons of the supraoptic, circularis (Tweedle and Hatton 1976; 1977), and paraventricular nuclei (Gregory and Hatton 1979). This is "dose-dependent," in the sense that more dehydration produces more glial retraction and has the net effect of leaving the neurosecretory cell bodies in exceptionally close (65–70 Å) soma-somatic apposition. Rehydration of the animal results in re-insertion of these processes between the neuronal parakarya. Under similar conditions of dehydration, the vasopressin- (antidiuretic hormone) containing cells of these nuclei undergo alterations in their electrical activity. Normally these cells fire at rates of 0.5 to 5 spikes/second and show no particular pattern of discharge. With dehydration, rates of up to 20 spikes/second occur in a phasic bursting pattern, where bursts and interburst intervals may last for a minute or more. As with the glial process dynamics, rehydration re-instates the normal firing mode. Since these types of activity can be observed in 400–500 μm thick slices of hypothalamus (Hatton, Armstrong, and Gregory 1978), one may hypothesize that synaptic mechanisms may not be involved. Such modulation of the pattern of activity (which, incidentially, is crucial for maximizing hormone release) may be due in large part to periodic extracellular $[K^+]$ increase in the 65 Å cleft between neurons. In such a restricted space K^+ could quickly reach levels that are more than twice the normal extracellular concentration, causing membrane depolarization and spike failure, thus terminating the burst until the membrane is repolarized. This high extracellular $[K^+]$ would also be capable of increasing the metabolic rate of the bursting cells (Lipton and Heimbach 1977), a desirable consequence under circumstances of increased hormone transport and release.

The axonal terminations of many of thse magnocellular neurosecre-

tory neurons are in the posterior pituitary, where there exist glial cells that are modified astrocytes, called pituicytes. These, too, may be able to modify the activity of the cell processes with which they come in contact, by engulfing the processes and thus altering the characteristics of the extracellular space surrounding the neuronal endings (Tweedle and Hatton 1979a; 1979b). The degree to which these specialized glial cells interact directly with the neurosecretory processes is quantitatively related to the animal's hydrational state. More pituicyte engulfment of neurosecretory processes occurs when the animal is well-hydrated. Dehydration produces a progressive disengagement of pituicytes and neurosecretory endings. These released endings are then presumably free to secrete their products into the perivascular space surrounding the fenestrated capillaries of the neurohypophysis.

These two examples of neuron-glial cell interaction and possible role in modulating neural activity may be unique to the system described, or they may be of more widespread significance. It is interesting to note that hippocampal pyramidal cells show close soma-somatic appositions (Green and Maxwell 1961), at least under some conditions. Until more work is done on the dynamics of hippocampal astrocytes, one can only guess at what functional significance such small interneuronal clefts might have in a structure so prone to seizure activity. Recent work indicates that the glial cells are involved in this activity (Schwartzkroin and Prince 1979).

I have given these examples in order to point to what appears to be another dimension of nonsynaptic neural modulation. Also, I felt the need to enter the plea: don't forget the glia!

by **Graham Hoyle**

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Classification of communications between neurons. In his target article Dismukes provides us with a useful summary of recent papers on various chemical aspects of neurophysiology and pharmacology, almost entirely from work on vertebrates published in 1976, 77, and 78. The only specific comment I wish to make is that one type of event postulated in vertebrates is now firmly established in an invertebrate. The abstract states: "it remains to be established . . . that complex electrophysiological effects are associated with substances released nonsynaptically." There is now one example in which several such effects have been established, namely for actions of octopamine released nonsynaptically by an identified locust neuron, DUMETi, (Hoyle 1975; 1978a; 1979). This neuro-active substance (NAS) affects the membranes of relatively distant, noninnervated muscle fibres (Hoyle 1978b), and it has a dual presynaptic action on equally distant motor nerve terminals (Evans & O'Shea 1977) so it is clearly acting as a modulator [see *Evans*].

Dismukes's principal purpose is to seek a possible classificatory framework, preferably a natural one, that incorporates common ground in definitions of descriptive terms that have become fashionable. He does not provide a comprehensive report, but instead he uses a piecemeal approach, picking about in the literature. One leaves this article with a strong feeling that a logical, *a priori* system is needed at this time. In thinking about the range of currently known or guessed neurochemical actions, which may be but a fraction of the actual number, I was reminded of a chart called "Radiation" that used to hang on a wall of the physics laboratory in my grammar school. It outlined the full range of known wavelengths of a scale from shortest to longest, pointing out major interesting properties in certain ranges. There were γ rays, x rays, wireless waves, heat rays, ultraviolet, visible spectrum, etc. Certain tantalizing gaps existed where, it was hinted, future research might detect unknown natural forms like the "healing" rays of homeopathy and death rays of science fiction. Unfortunately, merely knowing the wavelength tells one very little – and nothing at all about the really interesting qualities of actions associated with it. Prediction of these qualities is absolutely impossible, even if given not only the wavelength but other significant parameters of the radiation, such as the energy level. The reason is simple: there is nothing inherently special about a wavelength. Ultraviolet wavelength is invisible to humans but very significantly "colorful" to bees. In humans it can play havoc with skin, setting in motion events ranging from pigment production to cancer. The purpose of the radiation chartist seemed clear to me at the time, and the chart was certainly mnemonic and stimulating, but it was not good physics because of the absolute dependence of the effects produced on the qualities of the responding systems, which ranged from iron to eyes. It is the same with neurochemical signals. Allowing for secondary actions and interactive feedback from the initial effects, a vast range of phenomena is encompassed, from the simplest reflex to abstract thought.

The physicist who drew up the chart had the advantage of at least one common variable and was therefore able to clasify ultraviolet light solely by its wavelength. This would-be classifier of neural phenomena cannot find a single common variable in neurochemical communication. I tried doing it by time scale of the neural events, and this worked fairly well at the short end of the range, where molecular and bioelectric events are the goals of the communication. But even in the millisecond range there are many different kinds of molecular action, most of them poorly understood, including configurational shifts, gross molecular structural re-organizations, molecular incorporation or loss, charge movements, proton transfer, water molecule alignment, and so forth.

Next in the time scale come actions involving ion channels or ionophores. Currently well-documented events are those in which current flow of a particular ion species are known from voltage-clamp studies. These initial actions may not, however, be the ultimate purpose of the signal. The altered flow of ions, once started, must, in natural conditions, lead to a wide range of secondary events. Furthermore, the changes themselves are time-dependent as well as voltage-dependent in complex and interactive ways. The time-scale base for classification fails to be useful as soon as the overall biological effects are considered. An initiating event lasting for but a few milliseconds may have effects lasting for the rest of the lifetime of the animal.

Anatomical bases. The next thing to try is anatomy. Some degree of anatomical reference is essential in all biological descriptions, but a strictly anatomical basis for classification turns out to be inadequate in neuroscience. Not only can a wide variety of events occur at a single, precisely-defined small location such as a patch of postsynaptic membrane, but conceptually very different, even opposite events may also occur at the same anatomically-defined sites.

Chemical substances released by neurons. The next base to try is chemistry of neuroactive substances (NAS). Neurons release a wide range of substances that must initially be byproducts of the cell's metabolism. A very few of these by chance have consequences of positive survival value and eventually come to be released in a systematic way. Dismukes introduces the biological imperative in his definition of NAS (which he calls neurohumors). Once a NAS has become a regular product of neural activity, the way is opened up for the evolution of other forms of sensitivities to it, then for the evolution of other agents acting on the new sensitive spots. As quantities of NAS increase, stages in its production become available for the acquisition of roles also. We have, until quite recently, been in the habit of thinking that a very small number of substances are purposefully released by neurons, but their number is now growing rapidly and nobody in the field would care to hazard a guess as to what the final number will be. Neuroscientists would no longer be surprised by the suggestion that there are several hundred.

Chemists will always first try to classify on the basis of molecular configurations, but it is already obvious that this will not work for us in neurobiology. The physiological actions of a single NAS are in some cases very different at different targets, while similar actions can be produced by different NAS's. Broad classes such as biogenic amines and neuroactive peptides are useful as chemical classes, but they fail to conjure up images of particular actions – molecularly, physiologically, or behaviorally. All the indications point to there being no simple relation between chemical configuration and action.

Types of event in NAS interaction. Finally, we come to try a functional approach, first seeking to list the known functions and then seeing if they fall into clusters. Here we meet with a new set of difficulties. The final goal may be a brief membrane potential decrease; but should this be considered without reference to the site at which it occurs, or the processes that lead to it, or even the sites and mode of release of the

NAS? Isn't it important, also, to know if the action is solely at the surface membrane, or involves intracellular events? Suppose it is associated with calcium entry and release of cAMP? Shouldn't these be implied to be involved by suitable subcategories of the classification? And what of modulation? The biophysical events and anatomical sites in a modulation may be identical to those occurring in a primary, or direct event, but the functional intent is quite different and warrants separate treatment.

Dismukes claims that popular usage has adopted the term modulator "to characterize actions . . . which . . . elicit unconventional responses." The word has, in fact, a quite precise meaning, which is most readily seen in the field of music or, better still, in radio transmission. It relates to small variations in either amplitude or frequency. There are many other uses of the term in physics, such as phase modulation, but they can all be reduced to the amplitude/time domains. The intent in neuroscience is clearly the same: to convey that the basic effect (the direct-action NAS) is not rigidly fixed but can be increased or decreased somewhat or altered in time by the action of a secondary (modulatory) NAS. The latter can, as in presynaptic inhibition (whose purpose is clearly modulatory), be of a nature exactly similar biophysically to the primary event. The suggestion of "unconventionality" as the criterion therefore cannot be accepted.

In physiological function the term modulation can be given exactly the same connotation it has in physics. In most known instances the effect is on the amplitude of the initiated event. In others it is on a pacemaker or neural network, where frequency of discharge of individual neurons is altered, changing overall frequency or pattern. These classes of action can be usefully segregated, regardless of the physical nature of the sites involved, the type of NAS that causes them, or the time-course of the modulatory action. Eventually one settles for some form of mixture of biologial purpose, immediate function, gross anatomical features, ultrastructure, release process, reaction events, and chemical nature of the NAS. I have set out such a scheme below.

Descriptive terms. There are few terms that I find totally unacceptable, but one of them is humor. If Aristotle ever went off the rails in his bid for the use of logic in approaching nature, it was in his humoral theory. Nurtured by Galen and medieval philosophers, this absurd deterministic notion, intrinsic to the humoral theory, has been a scourge of mankind for more than 2000 years, and I hope not to be expected to have to perpetuate it. I greatly prefer the noncommittal term neuroactive substance(s), (NAS) which I shall define as: *a substance emanating from a neuron that affects a neural property or function.* All NAS's are, by definition, *transmitters* of information, so we can dispense with the word transmitter as being superfluous.

Towards a comprehensive classification. Are we to classify using underlying biophysical events, or their biological consequences? Evolution can act only upon extrinsic, behavioral aspects of the consequences. It is an additive process at the biophysical level, that cannot go back to make a fresh start. For example, having discovered inhibition and how to make use of it, Nature later needed to discover how to inhibit the inhibition, and so forth. A "natural" taxonomic classification attempts both to understand the evolutionary process and to follow it. A physiological classification should do no less, if it can, so a good starting point is to make biological purposes the first consideration. There are many such for a NAS, but they all fall into one of two categories: they are either for *direct action,* themselves causing functionally significant events, or for *modulation,* serving to change a direct action quantitatively or temporally. I deem it important to make this simple distinction a first basis for classification. This is a major exception to Dismukes's approach, in which modulation is included in the broad class of "synaptic neurotransmitter" action. Doubtless there are modulators of modulation, or NAS's that have a direct action on one system and a modulatory one on another, requiring dual categorization.

Subcategories of primary NAS actions must be classified arbitrarily, but using as many as possible of the major associated characteristics. It is probably best to start with anatomical ones, the most obvious being the synapse, dealing with the pre- and post-elements separately.

Table 1. (Dismukes). *Classification of chemical communication between neurons*

Class I. DIRECT ACTION. The neuroactive substance (NAS) initiates a behaviorally significant function.

Class II. MODULATION. The NAS alters the amplitude/time relations of a neural action.

A. *Reflexive modulation.* Modulation that is a standard part of the behavioral repertoire which is altered only temporarily by its action.

B. *Learning/memory.* Modulation that results in the establishment of a memory trace or of conditioned behavior.

In each Class there are common types of biophysical events, as follows:

1. SURFACE MEMBRANE ACTION. The NAS binds to a component of the membrane, usually for the purpose of inducing an electrical potential shift.
   a. *Conductance changes.*
      i. Local postsynaptic (PS). (classical synaptic transmission for Class I, presynaptic inhibition for Class II). Conductance change in a postsynaptic membrane caused by NAS focally released from an anatomical specialization (commonly a presynaptic terminal), interacting with receptor molecules.
         a) With local enzymic degradation of the NAS. Action in the millisecond range.
         b) Without local enzymic degradation of the NAS. Action from a few milliseconds to several seconds.
            i) without sequestration of NAS (longer lasting).
            ii) with sequestration of NAS.

General subcategories for each of the above include (all either slow or fast): Excitatory (E), Inhibitory (I), EL, IE, EE, II; depolarization of long duration (DL), polarization of long duration (PL)

      ii. Non-local postsynaptic. Changes in ion conductance at postsynaptic membrane sites other than those immediately underlying the release sites.
      iii. Nonsynaptic. Changes in membrane conductance brought about by NAS at nonsynaptic sites. These will particularly affect frequency (in pacemaker neurons) and pattern (in bursting neurons).
   b. *Enzyme activation.* Enzyme actions (other than those involved in ion pumps) that are promoted by NAS. Not yet positively established, but a possibility for adenyl cyclase.
      i. Postsynaptic. Enzyme is located in postsynaptic membrane. A wide variety of secondary events, depending on the action(s) of the material (e.g. cGMP, cAMP) synthesized by the enzyme.
      ii. Nonpostsynaptic. Same as postsynaptic but at nonsynaptic sites.
   c. *Ion pump activation.* Membrane ion pump acceleration or suppression by NAS
      i. Postsynaptic.
      ii. Nonpostsynaptic. (Subclassification by action on membrane potential or specific ion if known).
   d. *Molecular events other than a–c above.* A large category covering anticipated discoveries of actions at the membrane level affecting:
      i. Passive electrical properties. Membrane molecular configuration (resistance and capacitance), molecular incorporation, state of water or lipid, etc.
      ii. Dynamic electrical properties. Thresholds and time-relations of voltage-dependent ion conductances, etc.
   e. *"Recognition" of NAS by membrane-located molecules that are linked to gene expression.* Many follow-up effects as subcategories.
2. ENDOGENOUS ACTION. Actions of a NAS that ocur within the neuron following uptake by endocytosis.
   a. *Internal membrane effects.* Action(s) of the NAS at internal membrane(s) of: mitochondria, endoplasmic reticulum, nucleus, Golgi body, vesicle, etc.
   b. *Nonmembrane-related effects.* Action(s) of the NAS at sites other than membranes. Many alternatives including: active transport system(s), enzymes, growth areas, nucleic acid information processing.
      i. Presynaptic. Well-known effects include:
         a) Facilitation. Progressive enhancement of event.
         b) Antifacilitation. Progressive diminution of event.
      ii. Nonpresynaptic. Trophic control of neuron – general maintenance and characteristics qualities.

Yet is it not more fundamental to first separate surface membrane actions in general from intracellular events, regardless of specific anatomical features? I came to the conclusion that it is. The surface membrane events are by far the better known, but the endogenous ones are also very important, and some of them are of a nature markedly different from any membrane-associated action. Nowhere in Dismukes's article is there a term that relates to the important class of actions that occur endogenously. Indeed, this points to a weakness in his definition of "synaptic neurotransmitter" which "is mediated through membrane receptors."

In thinking about molecular details of surface membrane interactions, one immediately realizes that there are basic difficulties in definition, even of the membrane. Just exactly what is included in the term? Should it be restricted to the basic structural elements, the lipid bilayer, and associated binding proteins? Or should we include pores and ionophores (if these are distinct from structural pores)? Does it include ions and larger molecules, including enzymes, that happen to adhere to, or be attached to, the membrane, but are not incorporated in its structure? Then, in thinking about endogenous action, should one separate, in principle, actions that occur at internal organelle membranes, of endoplastic reticulum, mitochondria, and Golgi bodies, from those occurring at unattached free molecules or particles? What about actions on membranes surrounding vesicles inside the neuron?

All the above applies to consideration of actions occurring near the site of release of NAS. What is the best way of handling actions taking place at more distant targets after diffusion or circulation, or both? Dismukes uses the term neurohormone to denote those substances that "act on multiple and distant target cells" regardless of the type of action they produce here. A NAS that acts on multiple, distant neurons may, however, also have similar or different actions on nearby neurons. They key quality of a traditional neurohormonal action is the overall biological function that comprises a complex mixture of specific components with a slow, long-lasting time-course. Very little is known yet about the cellular details of action of undoubted neurohormones on nerve cells and circuits. Some are direct, others modulatory, and there are probably mixes of the two basic actions within some, or all. Therefore, neurohormonal action does not fall within the purview of classification at the level of interest of either cellular processes or functional intent. The terms of reference for a hormone are, by definition, that it serve as an "arousal" agent and be secreted by ductless glands into interstitial fluid or blood. The fact that some hormones are produced by neurons to act on neurons, or have actions on neurons as well as other types of cell, is accommodated in the general scheme proposed below, but this scheme does not recognise this term as a distinctive classificatory unit. Some neurohormone actions are known that are on surface membranes and others that are endogenous.

A newly-recognized event is uptake of released substance by the releasing neuron. This seems absurd, but it may be that the same substance is differently available, and therefore differently effective, after being taken up endocytotically, than when it is itself awaiting release. For this, how about autosynaptoendocytosis: a subcategory of neuroendocytosis?

Within each of the major subdivisions I have used either anatomical or biophysical characterizations for final classification.

As to whether or not the effort to make such classifications is worthwhile, there can only, in the long-rung, be an affirmative answer if it survives, but one will survive only if it is useful. Dismukes defends the practice by saying that it may have heuristic value. That was certainly the case with the radiation chart, which I still remember quite accurately from wall-gazing forty years ago.

by **Masao Ito**

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What is the primary contribution of the proposed types of communication to neuronal networks? I appreciate very much the great effort by Dismukes, who has explored vigorously the possible new types of communication among neurons, intermediate between classic synaptic transmission and hormonal remote regulation. I agree entirely with the author in recognizing that these new types of neuronal communication constitute a new field of brain sciences, and I feel in sympathy with him in many points of arguments raised in his target article.

One point, however, on which my feeling may be different from the author's is the degree of expectation as to how much the brain owes to these new types of neuronal communication. The second to last sentence of his article gives me the impression that Dismukes believes that these types of communication convert a neuron from a black box to a pretty sophisticated computer. How can such a magic power be lent to a neuron by a less specific, more diffuse, and more tonic sort of influence, which may add to classic synaptic transmission? I would like to hear more concrete ideas from the author as to what could be the primary contribution of the new types of communication to the functions of neuronal networks in the brain.

Of course, Dismukes does discuss one possible behavioral aspect at the end of the article, but I would like to point out another category of function which may be relevant to the new type of communication. There has been no good evidence indicating that either a classic synaptic transmitter or a neurohormone is responsible for the development of specific connectivity in neuronal networks and their maintenance, repair, or modification. It seems important to consider the postulated new types of communication in connection with these kinds of "house-building and house-keeping" jobs, so to speak, rather than specific information-processing, which we may conceive in our mind in terms of a sophisticated computer. These jobs are something like the wiring, stabilization, repair, or partial replacement of a computer, and one does not usually think of them in terms of information-processing. The recent elegant work by Kasamatsu and Pettigrew (1979a; b) may provide an example of such a maintenance function, as the locus coeruleus noradrenergic system is shown to be essential for plastic modifiability of visual cortical networks.

A rather minor but important point may be raised concerning the beginning of the seventh paragraph of the section entitled *Complex electrophysiological responses to neurotransmitters*. Even though I understand the possibility that some transmitters can modulate the efficacy of transmission at other synapses *temporarily*, I do not see why this implies anything relevant to memory and learning. Just a heterosynaptic interaction is not enough to account for memory and learning, unless the interaction is of a plastic nature. I am afraid that the author's statement here involves an overemphasis on the type of neuronal communication dealt with in this article.

In connection with the lovely section concerning intracellular transport of nontransmitter molecules, I would like to mention our recent finding that axonal flow from the inferior olive through cerebellar Purkinje cells plays an important role in maintaining inhibitory synaptic transmission from Purkinje cells to their target neurons – for example, Deiters neurons. Chemical destruction of the rat's inferior olive with 3-acetylpyridine leads to the disappearance of Purkinje cell inhibition in Deiters neurons (Ito, Orlov, and Shimoyama 1978). In rabbits, reduction of Purkinje cell inhibition of Deiters neurons was shown to develop rapidly, reaching a plateau within five hours after electrolytic destruction of the inferior olive (Ito, Nisimaru, and Shibuki 1979). Since inactivation of the inferior olive by local application of tetrodotoxin did not mimic such an effect, and since impulse propagation in Purkinje cell axons remained unaffected, it is quite likely that the effect of the olivary lesion is transferred to Deiters neurons by a nonimpulse process, presumably by axonal flow along olivocerebellar climbing-fiber afferents and Purkinje cell axons. This phenomenon would be an interesting example of remote communication within a neuronal network mediated by axonal flow, which serves for dynamic maintenance of synaptic transmissibility.

by **Leslie L. Iversen**

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Co-transmitters, modulation, and the peripheral nervous system. Dismukes has done a valuable service in producing a balanced review of recent developments in concepts of chemical transmission in the nervous system, and I found little of substance to disagree with.

The possibility that chemical transmission may take place between CNS neurons without morphological synaptic specializations comes as no surprise to those of us who were brought up with the autonomic nervous system as our model. In the sympathetic and parasympathetic nervous systems the axon terminals that secrete norepinephrine or acetylcholine rarely if ever make conventional synaptic contacts with effector tissues, and the released transmitters may have to diffuse for distances of several micrometers to reach their targets. Furthermore, there is ample evidence that the sympathetic and parasympathetic terminals interact; released acetylcholine inhibits the release of norepinephrine from sympathetic terminals, and vice versa – again without any specialized presynaptic axo-axonic synapses. In the autonomic nervous system acetylcholine and norepinephrine have rightly been viewed as neurotransmitters, despite the absence of specialized synapses. If we now find that neurons containing norepinephrine and other monoamines in the CNS similarly release their transmitters in areas where no synaptic specializations are present, this is perhaps what might have been expected from previous knowledge of the nature of such neurons in the periphery. To turn the argument around, what is surprising about the arrangement of monoaminergic neurons in the CNS is that they *do* make a rather large number of specialized synaptic contacts with target cells. What advantage can there be in such a mixture of "point-to-point" contact and diffuse release of transmitter from nonsynaptic zones, and what is it about certain target cells that induces specialized monoaminergic synapses to form during development? I would certainly agree wholeheartedly with Dismukes that chemical messengers that are released nonsynaptically should not necessarily be regarded *de facto* as belonging to some special category such as "neuromodulators." The terms "synaptic neurotransmitter" and "nonsynaptic neurotransmitter" seem useful and adequate descriptions.

The discovery that neurons may contain more than one biologically-active substance is an important development. The co-existence of substance P with 5-HT in some, but not all, of the raphe serotonin neurons has been confirmed by Hökfelt et al. (1978), and these authors have also reported that other neuropeptides such as somatostatin can co-exist with norepinephrine in adrenergic neurons in sympathetic ganglia (Hökfelt et al. 1977; Emson 1979). It is still unclear whether in such neurons the "co-existence" of neuropeptides with conventional monoamine transmitters in the neuronal perikarya necessarily implies that both materials occur together and are released from nerve terminals, although this seems entirely possible. The biological significance of the phenomenon, however, remains completely obscure. Using the term "co-transmitter" to describe such an eventuality may seem appropriate.

Dismukes's discussion of the ill-defined concept of neuromodulation and "neuromodulators" is particularly timely. The term neuromodulator has suddenly become fashionable and is often used gratuitously in the current scientific literature. One sometimes suspects that the most common usage is to describe a "preputative" transmitter status for some new brain chemical – to describe the possible function of such a compound as a neuromodulator is sufficiently vague to protect the author from possible recrimination that might come from the use of the more precise term neurotransmitter. Kupfermann (1979) has recently provided a thoughtful review of the modulatory actions of neurotransmitters, and like the present author he correctly views neuromodulation in terms of the type of interaction involved, rather than in terms of a special class of chemical messengers, the neuromodulators. In his view the most common attributes of modulatory synaptic effects are long duration of action and contingent action. Contingent action refers to the property that modulatory transmitters often have little or no effect in themselves, but instead they alter the effects of other events. Interest in such phenomena is heightened because the long duration and contingent nature make modulatory effects ideally suited to the control of behavioral modulations such as learning, motivational state, arousal, and sensitization. In addition to the examples of modulatory phenomena quoted, one might add the finding by Belcher and Ryall (1977) that substance P acts on Renshaw cells in the spinal cord to antagonize the nicotinic actions of acetylcholine, while having no effects when administered alone. It is possible that neuropeptide actions in the CNS may be largely of such a modulatory character, although not enough examples have yet been studied.

The use of chemical messengers in the nervous system clearly extends beyond the point-to-point system of synaptic contacts and rapid "on" and "off" actions with which we are familiar. However, the system of visible synaptic connections between neurons in the CNS is already complex enough to defy comprehension – with billions of synapses per gram of cerebral cortex. To add the dimensions of nonsynaptic transmitter release, co-transmitters, and modulatory actions will probably take some decades for adequate digestion.

by **Irving Kupfermann**

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Thank goodness we do not need a definition of modulation. Dismukes says it is intriguing to speculate that behavioral modulatory functions (such as arousal) might be mediated by modulatory effects at the cellular level, and specifically that serotonin might "have global effects on behavioral state." He states that there is little evidence for such confluence. Furthermore, he feels that the uncovering of such evidence is probably dependent upon first finding more explicit notions of modulation. This appears to be a rather pessimistic view, given his own cogent arguments against explicity defining modulation. Fortunately, however, there is substantial evidence that there is indeed a confluence of behavioral modulation and neural modulation.

The evidence has been obtained largely in invertebrates, but it clearly illustrates that, in principle, progress can be made even in the absence of an explicit definition of neural modulation. Thus, for example, in *Aplysia,* two different forms of behavioral modulation critically involve modulatory synaptic actions. The behavioral modulations are: 1) sensitization due to a strong noxious stimulus (Kandel 1978), and 2) behavioral arousal due to exposure to an appetitive food stimulus (Kupfermann, Cohen, Mandelbaum, Schonberg, Susswein, and Weiss 1979). Both sensitization and food-arousal in *Aplysia* involve synaptic actions that would be called modulatory according to almost any definition. Sensitization is due to a presynaptic action of serotonin (or related amine). The transmitter (or modulator) affects voltage-sensitive ionic conductances that result in enhanced calcium influx and increased transmitter release at the terminals of sensory neurons (Brunelli, Castellucci, and Kandel 1976; Klein and Kandel 1978). The effects appear to be mediated by cAMP. Food-arousal in *Aplysia* is mediated by a serotonergic neuron that has numerous central as well as peripheral actions (Weiss, Cohen, and Kupfermann 1978). All of the actions are very slow in onset and decay. The peripheral effect of serotonergic transmission consists, in part, of an enhancement of contractility of muscles involved in biting responses. Several lines of evidence indicate that this action is mediated by cAMP and apparently does not involve any alteration of membrane conductance or potential (Weiss, Mandelbaum, Schonberg and Kupfermann 1979).

Thus, although an explicit definition of modulation would be desirable, in the absence of such a definition, meaningful communication between workers is possible, and substantial experimental progress can be made. The history and usage of the term neuromodulation is distressingly similar to that of the command neuron (Kupfermann and Weiss 1978). Neuromodulation, however, seems more benign, perhaps because, unlike the command neuron concept, it is not quite so loaded with implications concerning normal behavioral function.

by **F. Lembeck**

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Defining neuromodulation. The article of Dismukes offers a broad

discussion of the communicating systems among neurons beyond the old-fashioned neurotransmission, such as neuromodulation, and so on.

What modulation is seems to be generally known, because the radio has knobs for FM and AM. The British Encyclopaedia defines it as "the process of varying the frequency, amplitude, intensity, or phase of a carrier wave so as to conform with a transmitted signal." The CNS is even more complicated than a radio; why shouldn't it also use modulation? Modulation can easily be observed neurophysiologically. The "very simple" monosynaptic reflex is already modulated by a considerable number of additional neurons impinging on the approximately 200 "plugs" of one motoneurone. All these neurons communicate by the mechanism of *neurotransmission,* and the *result* of this communication is seen as a *modulation of the reflex.* Modulation of neural communication may also result from different extraneural influences: pressure causes a frequency modulation in baroreceptor fibers; stimulation of pain receptors is modulated by PGE_1; light modulates the nervous inflow to the muscles of the iris. Again, modulation is, in my view, always a *result,* produced by various and different mechanisms.

Can we *call* a substance a "neuromodulator," "neuroregulator," and so forth? Certainly we can, just as we use the term "meat tenderizer" for an enzyme scientifically named papain. Acetylcholine has been agreed *to function* as a neurotransmitter. Acetylcholine, present in the nerve-tree placenta, will not, however, have such a function there. *Lactobacillus* supplies sauerkraut with a high content of acetylcholine: Is acetylcholine therefore a "sauerkrauter?" Similar absurb examples could be shown with the catecholamines, histamine, or serotonin.

Definitions in biology are as good as traffic signs if they are used to clear a situation. Definitions have the danger of becoming philosophical headlines, keeping experimental workers busy trying to fit their experimental facts to the theoretical definition. Definitions can certainly be used for an intellectual approach in order to explain or discuss well-defined experimental facts. Sir Henry Dale, once looking at a rather imperfect kymograph tracing, said "Nothing depends on curves, all depends on the reliability of the observer." The "curves" in the article of Dismukes are neurohormones, neurohumors, neuromodulators, and neuroregulators, and the careful description of their basic properties is undoubtedly most stimulating.

If the reader should get the impression that my commentary is very vague, than he has exactly pinpointed my view. "It will probably always be more important to try a thing out than to argue about it," said Sir John Gaddum, and this should be the coming procedure here, too.

by **B. Libet**

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Neuronal communication and synaptic modulation: experimental evidence vs. conceptual categories. The paper by Dismukes is a worthwhile and timely examination of conceptual possibilities raised by an increasing variety of experimental observations that are not compatible with current classical views of synaptic transmission. Indeed, our own findings on the existence and nature of a variety of slow synaptic actions in sympathetic ganglia provided some of the first synaptically defined models and proposals in this direction (Libet 1965; 1970; Libet and Tosaka 1970; Libet, Kobayashi, and Tanaka 1975). It is perhaps not surprising that in an area that exhibits potential for major developments in the understanding of important functions of the brain, there should have arisen a considerable amount of overextended speculation as well as looseness of concepts and terminology. Dismukes does sound some warnings on this score. But many of the hypotheses and concepts described are, in my opinion, not subjected to sufficiently critical analysis and clarification in relation to the experimental evidence. I would therefore like to 1) comment on the relations between the suggested modes of chemical communication and the available lines of experimental evidence from which the suggestions derived; and 2) consider the adequacy of the nomenclature proposed by Dismukes for dealing with the prevailing definitions and concepts, and to offer an alternative approach to systematizing the issues in chemical communication among neurons.

Experimental evidence and concepts of chemical communication. One major line of evidence comes from morphological and histochemical studies, which strongly suggest that at least several monoamine transmitters may be released "nonsynaptically" in the brain. As an alternative to the term "nonsynaptic" I would propose that of "loose synaptic"; the latter would then contrast to the "specialized close synaptic" arrangement of the "classical" chemical synapse (see Libet 1965; 1976, 1979b). In this way the broader term "synaptic" coupling would cover both forms, "specialized" and "loose," and could retain its broader functional meaning; limiting the term synaptic to those cases that possess the special ultrastructural features associated with one general group of classical postsynaptic potentials (PSP's) would introduce an unnecessary constraint on functional views of neuronal couplings with different structural features. (Incidentally, the long synaptic delays for the slow PSP's in sympathetic ganglia, and the even longer time [30 sec or more] for induction of the modulatory action by dopamine (DA) provides a physiological basis for release of transmitters at some distance from receptor sites, in addition to morphological evidence [Libet 1967; 1979b].)

If "loose" or "nonsynaptic release does occur, certain inferences about spatial and temporal diffuseness of postsynaptic actions could be proposed along the lines of Dismukes's first section. But it should be recognized that such features in themselves cannot be taken to imply neuromodulatory or neuroregulatory roles. The same sort of diffuseness of action could be achieved, for example, by having the multiple, classically-specialized synaptic endings of a given presynaptic axon distributed diffusely over a postsynaptic unit. Neither this, nor – as Dismukes points out – the apparently loose or nonsynaptic release and diffuseness of action of norepinephrine at peripheral effector sites, would be taken to qualify for a modulatory rather than the usual transmitter role. Modulation or regulation, to have a more distinctively useful meaning, would appear to require a qualitatively different kind of action, not simply a spatial or temporal diffuseness of postsynaptic effect by the transmitter.

Another major source of evidence derives from "complex electrophysiological responses to neurotransmitters." The first and perhaps best studied examples of this in the vertebrate nervous system have in fact been the slow inhibitory and slow excitatory postsynaptic potentials (s-IPSP's and s-EPSP's) in sympathetic ganglion cells, the existence and extraordinary slow features of which were established in the 1960's (reviewed in Libet 1970). It was further discovered that both slow PSP's are generated without any increase in ionic conductance or membrane permeability, such as that which underlies classical "fast" PSP's and some other slow ones as well (Kobayashi and Libet 1968; Koketsu 1969). But the unique features of these slow PSP's (i.e., slowness and no conductance increases) do not in themselves impart a modulatory function to the s-IPSP or s-EPSP, though this has been suggested by some (e.g., Greengard and Kebabian 1974). Both of these slow PSP's have in fact been shown directly to influence the firing of intact postsynaptic neurons in the appropriate directions (see Libet 1970; when sympathetic ganglion cells, especially in mammalian ganglion, are impaled by a microelectrode for intracellular recordings, the slow PSP's are greatly reduced from their physiological values, apparently because of the almost inevitable cell damage and low resting membrane potentials of about −50mV, [See Libet 1970; 1979b]). There would, therefore, be no reason to regard their functions as anything other than those appropriate for inhibitory or excitatory PSP's, although of a slower variety and with electrogeneses different from those of classical PSP's.

The s-EPSP was found under certain conditions to be accompanied by a *decrease* in ionic conductance – i.e., an increase in membrane resistance, r_m (Kobayashi and Libet 1968; 1970). On the basis of this it has been proposed a) that the s-EPSP is generated by a decrease in K^+ conductance (see Weight 1974), and b) that the amplitudes of classical fast PSP's may be increased if they are developed during the course of a slow EPSP with an accompanying increase in membrane resistance (Schulman and Weight 1976). In the latter proposal, that of changing the postsynaptic responses to other presynaptic actions, the s-EPSP could be thought to play a modulatory role; Dismukes adopts and develops this line of approach. However, an increase in r_m is *not an*

essential feature of the physiologically generated s-EPSP; the increase in r_m has only been found when resting potentials of ganglion cells are depolarized down to −60mV or less (Nishi, Soeda, and Koketsu 1969; Kobayashi and Libet 1974; Hashiguchi et al. 1978), and it appears to be due to a different and nonspecific action of ACh (Kobayashi and Libet 1974; Hashiguchi et al. 1978).

One must, then, exercise caution in generalizing about the significance of proposed modulatory roles if these can appear only when cells are substantially depolarized; the possibility of such a qualifier being necessary, even for related examples of cerebral responses to acetylcholine or norepinephrine, may need further evaluation. (A decrease in conductance was also described for the s-IPSP in nicotinized frog ganglion cells [see Weight 1974b]; but the s-IPSP in the presence of nicotine includes a large "pharmacological" component that is qualitatively different from the physiological s-IPSP in the absence of nicotine [see Libet and Kobayashi 1974]; no increase or decrease of r_m has been found with the s-IPSP of curarized mammalian ganglion cells [Kobayashi and Libet 1968; 1970] or with the equivalent hyperpolarization by its direct transmitter, DA [Dun and Nishi 1974].)

There are, however, a number of other experimental examples of actions by neurotransmitters that are independent of the production of any PSP – i.e., of any direct change in level of excitation of inhibition by the transmitter. (Such actions I would regard as "neuromodulatory" – see below.) Among subcategories of this class is the "contingent action," in which the magnitude of PSP responses to one transmitter can be altered by a prior modulatory action of another transmitter, in a time-dependent manner. The "contingent action" obviously has great heuristic value in relation to many major brain functions, but the first demonstration of it in a defined synaptic pathway was made in the rabbit's superior cervical ganglion (Libet and Tosaka 1970; Dismukes cites a later similar proposal by Barker [1978] for a type of contingent modulatory action based upon certain actions of neuropeptides). Libet and Tosaka found that a brief exposure to the intraganglionic transmitter dopamine (DA) could induce an enduring increase in the magnitude of s-EPSP responses to acetylcholine; this could occur without any s-IPSP membrane response that is mediated by DA at other receptors on these same cells (Libet and Tosaka 1970; Kobayashi, Hashiguchi, and Ushiyama 1978; Libet 1979c). Additional evidence has indicated that this DA-modulatory action is mediated intracellularly by cyclic AMP, in a manner suggestive of processes that could lead to formation of a neuronal memory trace (Libet, Kobayashi, and Tanaka 1975; Libet 1979a).

Nomenclature proposed for modes of chemical transmission. The approach taken by Dismukes appears to center chiefly around the morphological features of the coupling between releasing sites and the locations of the target receptor sites where the chemical transmitter may act. This is certainly one worthwhile type of categorization, but Dismukes appears to confuse this issue by attempting simultaneously to associate these features with other concepts that are not necessarily related to them. Postsynaptic actions, including "complex modulatory as well as conventional excitatory or inhibitory ones," are listed under "neurotransmitter," as if this would provide a distinction from the "neurohormone"; while the "neurohormone" is said to be partly distinguished from the "neurotransmitter" by acting on "target cells which do not necessarily have membrane receptors or electrical responses."

It also seems questionable whether introducing the word "neurohumor" as "a generic term for any substance released by a neuron" will do more than add another cumbersome word to the lexicon in the field. As Dismukes himself recognizes, the crucial criteria formulated by Werman (1966) for identifying a neurotransmitter can apply to any mode of release or even of postsynaptic action.

Alternative general proposal for nomenclature and categories of modes of chemical transmission. Firstly, it would seem simpler to use the term "neurotransmitter," rather than "neurohumor," as the generic term for any substance 1) that is released by a neuron when electrophysiological membrane changes are transmitted to appropriate releasing sites; and 2) that alters the activity of other neurons, adjacent or distant. (This would not include the category of so-called "nontransmitter molecules" [Dismukes's section *Intercellular transport of nontransmitter molecules*], such as nerve growth factor, the "slow juice" of Eccles et al., by which slow motoneurones can change fast muscle fibers into slow ones, and so on.)

Secondly, the neurotransmitters and their various modes of action could be categorized according to a number of different issues that would not be mutually exclusive, rather than in one single form of nomenclature. I can list examples to indicate what I mean by this, without attempting to include all the current possibilities:

1. *Form and distance of the releasing site* in relation to the target receptor sites of the transmitter: the terms "synaptic," "nonsynaptic," and "neurohormonal" could be subsumed under this categorization, as in Dismukes's approach. Or, as I prefer, the terms "specialized synaptic" and "loose synaptic" could be substituted for the first two, respectively (see discussion above). In any case, the functional implications of this categorization would be restricted to those associated with this issue alone, such as delay in a postsynaptic reponse, diffuseness of effects, possible transport in vascular channels, and so on.

2. *Type of postsynaptic change induced by transmitter.*

A. *Electrogenic mechanisms* for the PSP's produced. Those mechanisms involving production of increases in ionic permeability by transmitters – as with classical fast PSP's and some slow ones – would be distinguished from those that are not based upon changes in ionic permeability, as with the s-IPSP and s-EPSP of sympathetic ganglion cells (see discussion above). It should be noted that there could, in principle, be a variety of electrogenic mechanisms, and that a difference between electrogenic mechanisms does not, in itself, necessarily imply any other qualitative differences in modes of functional significance between classes of PSP's.

B. *Duration of postsynaptic changes* (after arrival of the transmitter at the postsynaptic receptor sites). Again, relatively long-duration changes, compared to those for classical fast PSP's, do not in themselves require that they be defined as modulatory. For example, the s-IPSP, the s-EPSP, and the even more prolonged "late-slow EPSP" in sympathetic ganglion cells have durations in seconds to many minutes, rather than in tens of milliseconds (see Libet 1970; Nishi and Koketsu, 1968). Nevertheless, these slow PSP's can still share with faster PSP's the characteristics of direct excitatory and inhibitory functions. On the other hand, there may be long-duration postsynaptic changes, following a transmitter action, that are independent of any production of a PSP change in the membrane. For example, the enhancement in the slow muscarinic depolarizing responses to ACh (the s-EPSP response) that follows a brief exposure to DA can last for at least some hours (Libet and Tosaka 1970); —see, also, category C.

C. *Modulatory actions of neurotransmitters.* Although there is still no generally accepted definition of neuromodulation (Kupfermann 1979), I believe it is possible and presently necessary to make one that is inherently consistent, sufficiently distinctive, and useful. I would apply the term "modulatory" to all those actions of a neurotransmitter (as defined above), *other than those of direct excitation or inhibition,* which affect neuronal responses to other neural inputs (whether these involve the same or other transmitters). (To avoid over-eager speculation about modulatory functions, I would agree with Dismukes that particular transmitters and their apparent actions should not be labeled neuromodulatory or regulatory unless the specific system is identified.) The definition given here would correspond to that given for a "contingent action" in a broad sense, as expressed by Kupfermann (1979). But "contingent action" tends also to imply more specific couplings or interactions between one neural input and another, of kinds especially interesting to learning and memory as well as certain other brain functions. It may, therefore, be desirable to reserve the term "contingent actions" to describe one among a variety of possible types of neuromodulatory actions. These could include the following types: i) "Contingent actions," in which one transmitter, acting on an appropriate "modulatory" postsynaptic receptor site or mechanism, induces a subsequent change in neuronal PSP responses to another transmitter, normally delivered via a separate and different neural input (e.g., Libet and Tosaka 1970; Libet, Kobayashi, and Tanaka 1975; Barker, 1978 etc.). I would agree with Dismukes that one should speak

of the coupling between a specific receptor and a given tramsitter, since responses to the same transmitter can include a variety of general types, even for the same neuron (e.g., DA eliciting both direct inhibition and a modulatory change in sympathetic ganglion cells, Libet and Tosaka 1970). ii) Control of presynaptic release of transmitter, where this is accomplished with no PSP generated at the site of presynaptic release. iii) Induction of enzyme synthesis of transmitter by the postsynaptic unit, as well as other enzymatic changes of functional significance (e.g., the increases in tyrosine hydroxylase in sympathetic ganglion cells subjected to preganglionic input, as shown by Axelrod, Costa, et al.), and so on.

While such a multiplicity of categories may seem to be more cumbersome and complicated, it may have the advantage of greater flexibility and clarity about the different concepts and potentialities; it would also be in accord with Dismukes's own admonitions against attempting to assign overly general features on the basis of limited experimental evidence.

by **Sidney Ochs**

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Aspects of communication related to axoplasmic transport. Dismukes's review of new developments in our understanding of synaptic transmission includes interpretations based on the more recent findings of axoplasmic transport. The references to transport appear to be somewhat specialized, and the author might consider more general reviews – e.g. those of Grafstein (1977) and Ochs and Worth (1978).

In the section on Dale's Principle, the possibility that the same neuron may deliver different transmitters to the several branches of the same neuron is discussed. This may be explained by a recently considered aspect of transport, the phenomenon encompassed in the term "routing," where different amounts as well as types of materials are delivered to the various branches of a neuron (Ochs *et al.* 1978). As an explanation of routing, different sets of microtubules are considered to act as "rails" along which the transport filament mechanism interacts to carry the different materials into each of the nerve branches. By this means, different transmitters can be delivered to the individual nerve terminals. The question as to how routing is organized in the cell bodies to determine which materials enter onto the microtubules destined for their individual nerve terminals remains an interesting problem.

by **N. N. Osborne**

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Axonal varicosities, variable thresholds, and Dale's Principle. Dismukes presents a comprehensive discussion of the possible mechanisms of molecular communication between neurones. He points out, with justification, that overextending a general working principle can discourage the exploration of important exceptions and variations. This was a definite danger in the past, but with the increased opportunities for publishing individual ideas it has gradually diminished, as documented by Dismukes's article. There is still the risk, however, of authors' selecting ambiguous data from literature to support their arguments. For example, when discussing the validity of Dale's Principle, Dismukes states that Brownstein et al (1974) showed that several transmitters co-exist in certain *Aplysia* neurones, and he mentions, correctly, that I criticised the microdissection technique employed (Osborne 1977). What should also have been added is that attempts by other authors to confirm the data of Brownstein et al. (1974) have failed (e.g. Farnham et al. 1978; McCaman and McCaman 1978). Just how significant, then, are some of the data which shatter traditional concepts?

The nomenclature proposed by Dismukes for transmitters is simple and rather attractive. By placing classical synaptic transmitters, neurohormones, and apparent nonsynaptic transmitters under a single generic term (viz. neurohumour) a great deal of overlap is intrinsically implied. This would seem necessary to accommodate the various reports in literature.

The possibility of nonsynaptic release of substances is discussed in some detail by Dismukes, although I felt that the case for such a mechanism was unconvincing. Most of the argument is based on morphological data together with some autoradiographical results. For example, it is pointed out that aminergic axons have varicosities that contain the apparatus usually associated with storage and release of transmitters. Since these varicosities do not have classical synpases, it is argued that substances could be released from these sites and have their effects by diffusing to other cells – i.e. nonsynaptic release. However, the apparatus usually associated with storage and release of transmitters (viz. synaptic vesicles and mitochondria) is present in all parts of certain invertebrate neurones, including the somata (Osborne 1978). If there is a nonsynaptic release of transmitters, I see no compelling evidence that it is restricted to varicosities or to any other part of the neurone. The observation that when ^{3}H-noradrenaline or ^{3}H-serotonin is applied to the neocortex, it eventually accumulates within varicosities, is certainly a valid argument for the involvement of these sites in synaptic release, but not necessarily nonsynaptic release. After all, specific uptake sites are thought to be areas of the neurone involved in inactivation – i.e. at the synapse. In the case of nonsynaptic release, inactivation of the released transmitter will probably involve another type of mechanism, since the receptors would be a distance away from the release sites. Moreover, the autoradiographical data could reflect the results of a homoexchange mechanism between endogenous labelled transmitters, which is dependent on the endogenous concentration of substance. Since the varicosities contain greater concentrations of transmitter than other areas of the axon, they will have a greater exchange with exogenous radioactive substances.

I can only endorse the conclusion reached by Dismukes that nonsynaptic release of transmitters has not been established, although the idea fits in rather nicely with emerging concepts. There is, however, a danger in overemphasising certain concepts. The idea of dendritic (and cell body) release of dopamine from dopamine neurones in the substantia nigra, for example, is very recent and apparently accepted, although the evidence for such a mechanism is still incomplete. As an illustration, a prerequisite for dendrite dopamine release in the substantia nigra is proof that dopamine axonal synapses do not exist in this area. Whether the available morphological data demonstrate this unequivocally is, however, questionable.

It is also debatable whether the case in favour of nonsynaptic release is strengthened by the data showing that neurotransmitters produce in some neurones rather complicated responses that are not simply excitation or inhibition. The electrophysiological data can be interpreted in terms of normal synaptic transmitter release, and the apparent unconventionality of the responses as simply a reflection of the nature of postsynaptic receptors. We know that the overall electrophysiological effect of a transmitter depends not only on the nature of the substance but also on the nature of the postsynaptic receptors. In its extreme, a single transmitter (e.g. acetylcholine) released from a single cell may directly excite one neurone and directly inhibit another (see Gerschenfeld 1973). It is therefore possible that the postsynaptic receptors to a single transmitter may have a spectrum of configurations so that the threshold reached between the reaction times with the transmitter molecules can vary from microseconds to seconds. The threshold for reaction may also be dependent on the quantity of transmitter present. If this is the case, a number of the complex electrophysiological responses to transmitters may be explained in terms of normal synaptic release.

The validity of Dale's Principle has been the recent subject of wide discussion as a result of reports providing evidence for the coexistence of more than one transmitter in a neurone (Osborne 1979). In my opinion, the problem is first to decide which substances are actually transmitters. Substances such as ATP are present in all neurones and are known to be released from cholinergic and adrenergic neurones. Does this qualify ATP as a transmitter? It would be insufficient for the classical view of a transmitter whose definition involves interaction of the substance with specific receptors on the postsynaptic membrane, the reaction of which causes a change in the postsynaptic cell's activity. According to this definition, very few substances can be

regarded as transmitters, simply because of the difficulty entailed in demonstrating experimentally that they conform to our definition. The intriguing possibility that some neurones may utilise two or more transmitters as opposed to two or more putative transmitters will therefore remain an issue for many years to come. The difficulty of interpreting experimental results to accommodate our definition of a transmitter substance is a major problem in neurobiology and one acknowledged by Dismukes in his article. Creating new definitions does not simplify the situation unless the definition can be more easily tested experimentally. This will present a hurdle so long as the apparently necessary criterion remains of "a specific reaction with receptors."

by **Sanford L. Palay**

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Modes of interneuronal communication. Evidence has been accumulating for some time, suggesting that the well-known synaptic interaction between neurons provides only an incomplete description of the operation of the nervous system. In his summary of this evidence, Dr. Dismukes cautiously avoids making extreme statements or drawing startling conclusions. He also seems to understand that expanding the range of operational modes available to the nervous system neither invalidates the neuron doctrine nor diminishes the role of the nerve impulse or the chemical synapse. The operation of the nervous system is still conceived in terms of individual nerve cells and their parts, in terms of conductance and resistance to movements of ions, voltage differentials, and the interaction between transmitter chemicals and receptor molecules. Thus these "new concepts" and proposals are not so revolutionary as they may seem at first sight. It is not the neuroanatomists and the neurophysiologists who are scandalized by these "unconventional" proposals, but the neuron modelers and the information specialists, whose basic assumptions necessarily lead them into a rigid concept of the unitary nerve cell.

If we can find ways to verify these new modes of interneuronal communication, they may account for many properties of the nervous system that are difficult, if not impossible, to envision with a nervous system limited to the synaptic mode. Yet, in principle, there is no real difference between the local action of a chemical transmitter at a synapse and its more diffuse action in the absence of synapses. In both instances a chemical released from one neuron interacts with a receptor molecule on another neuron and the result of the interaction is a greater or lesser aleration in permeability, conductance or resistance, and voltage across the membrane. With currently available techniques we recognize the location of this interaction as a synapse when there is a discernible patch of specialized structure at the zone of contact between the two neurons. If no specialization is discernible, we cannot identify the site of interaction, and we do not recognize the contact as a synapse. Probably in some cases future advanced techniques will disclose the location of these now cryptic sites of interaction, and then we shall be able to say definitely whether those sites are local (synapses) or diffuse.

But there will still be places where synapses are not possible – i.e. apposition between the pre- and postsynaptic partners in the interaction. An example of this state is the intraventricular nerve fiber plexus that overlies the ependyma. In this case transmitter (serotonin) released into the cerebrospinal fluid must have diffuse access to receptors in the central nervous system. The resulting effects must depend upon the role of the transmitter-receptor complex in different sites, and there is no compelling reason to require *a priori* that these effects should all be uniform in all situations.

I do not share the author's concern over the varied use of the word "modulation" or his distress over what he considers the chaotic state of the nomenclature. The latter simply reflects the lack of well-established and well-understood facts with which to tether the various overlapping and sometimes conflicting speculations. But "modulation" is clear enough for present purposes. According to my favorite unabridged dictionary, to modulate means "to form or adjust to or regulate by a certain proportion; to temper, to soften." There is nothing in the definition that specifies mechanism, but the idea of tempering, adjusting, or tuning is the central theme. There is no reason at this stage to restrict our discussion by insisting on terms that specify mechanism, and no reason to expect that all modulations should use only one mechanism. The difficulties of such self-inflicted impediments to thinking are exhibited in Dismukes's own proposed classification of communicating molecules. His synaptic transmitter does not preclude "complex modulatory actions" and may be released at sites where the postsynaptic specialization may not be apparent, while his nonsynaptic transmitter, which could be the same chemical substance, must travel to multiple cells that can be distant from the release site. The differences between these two categories are obscure, since the same substance can be a synaptic transmitter, a nonsynaptic transmitter, and even a neurohormone, either at different sites in a single fiber or in different pathways; it will be some time before order prevails in the chaos that Dismukes fears.

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Neurotransmitters versus neuromodulators. Having defined a neurohumor as any substance released by a neuron to alter the activity of other cells (which, incidentally, would include K^+ and possibly Ca^{++}), Dr. Dismukes identifies three classes of neurohumors, namely synaptic (or junctional) neurotransmitters, nonsynaptic (or nonjunctional transmitters), and neurohormones. He further suggests that when the predominant effect of a transmitter substance in a particular system is regulation of the efficacy of other transmitter outputs or of the mode of operation of the target cell, then it may be useful to think in terms of modulation.

This classification, based on the proximity of the postsynaptic structure to the releasing terminal, tends to be ambiguous or leads to inconsistencies in some cases. For instance, in smooth muscle tissues, autonomic adrenergic terminals often do not form distinct synapses and release their transmitter in closer proximity to some muscle cells than to others. Although Dismukes's classification system distinguishes between the near and distant nerve junctions with muscle cells, the transmitter effect would be the same in both cases. Similar difficulties arise in the cerebral cortex where norepinephrine-containing terminals are relatively few in number, and are rarely associated with specialized regions of the postsynaptic membranes (Lapierre et al. 1973). In spite of this, 100% of the identified corticospinal neurons tested were inhibited by locus coeruleus stimulation (Phillis and Kostopoulos 1977), implying that the amine must diffuse extensively from its initial sites of release. Widespread diffusion, as a means of extending its effects after release from a limited number of terminals, would also explain the significance of findings that a high proportion of cortical neurons are inhibited by iontophoretically applied norepinephrine. The hyperpolarizing actions of applied norepinephrine are a result of stimulation of a membrane electrogenic sodium pump (Sastry and Phillis 1977; Wu and Phillis 1978). An important but unresolved question is whether norepinephrine elicits similar responses at both subsynaptic and nonsynaptic sites, or if it has different actions on the various receptors.

A simpler classification scheme for synaptic transmitters (Phillis 1978) might be to categorize them as *neurotransmitters* if they mediate transmission at rapidly-transmitting junctions (involving the nicotinic actions of acetylcholine, or the actions of L-glutamate, L-aspartate, γ-aminobutyric acid, and glycine), or as *modulators,* if they cause long-lasting alterations in neuronal excitability or transmitter release (muscarinic actions of acetylcholine, effects of adenosine, monoamines, and possibly peptides). Renshaw cells and sympathetic ganglion cells provide examples of neurons on which acetylcholine would have both neurotransmitter and modulator actions.

Dismukes does not delve into the distinct possibility that glial cells may be involved in the modulation of nerve cell excitability. Receptors for many synaptic transmitters appear to be present on glial cells, and their activation could ultimately lead to alterations in neuronal activity. For instance, glial cell Na^+, K^+-ATPase is stimulated by norepinephrine (Narumi et al. 1978), and enhanced K^+ uptake by glial cells, with an ensuing reduction in extracellular K^+ concentrations, would lead to neuronal hyperpolarization and inhibition. Conversely, agents that

inhibit K^+ uptake by glial cells would lead to an accumulation of extracellular K^+ and a consequent depolarization of neurons.

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What is a synapse? A discussion of the nomenclature used to describe communication between neurones is timely, in view of the explosion in the last few years of rather novel experimental data. In this sense the review of Dismukes serves a useful purpose in bringing into the limelight some of the difficulties of interpretation posed by the recent findings. However, I would find it extremely disturbing if his final recommendations concerning the division of neurohumours into a) synaptic (or junctional) neurotransmitter, b) nonsynaptic (or nonjunctional) transmitter, and c) neurohormone, were to be universally accepted at this stage. He argues for the retention of flexibility in nomenclature, but in the final analysis he proposes a scheme which, if accepted, will inevitably lead to confusion and ambiguity. I will argue the case for only two subdivisions into synaptic neurotransmitters and neurohormones.

The beauty of the English language is that it has a vocabulary containing words that convey a variety of shades of meaning, some encompassing a broader spectrum within their definition than do others. Let us therefore examine the meaning of the word synapse in the present context of interneuronal communication.

Synapse was originally coined by Sherrington to describe *functional* areas of contact between nerve cells communicating with each other. The word is derived from two Greek words simply meaning coming together. It is therefore quite appropriate that its meaning should have been extended from the limited case of nerve cells, proposed by Sherrington, to other sites at which nerves make contact with effector organs, as at the neuromuscular junction, and at which the physiological processes are similar. It is to be noted that this definition does not describe the synapse in morphological terms.

Morphological definition of synapses became possible with the advent of electron microscopy. The demonstration of the close apposition of two cell membranes provided an obvious anatomical basis for synaptic transmission. The apposition could be very close, amounting to fusion in the case of "tight" or "gap" junctions, and to less immediate apposition in more common junctions. The division of these into two functional types, as electrically or chemically transmitting, respectively, is not complete, because in the mature chick ciliary ganglion the diameter of the synaptic cleft would lead one to expect a purely chemical type of transmission, whereas in fact there is a mixture of chemical and electrical transmission due to the envelopment of the neurones by Schwann cells. Even at junctions where this particular complication does not exist, there is no single cleft diameter that is universal for a synapse, but rather there is a continuum of diameters, as pointed out by Dismukes. There is therefore no sharp dividing line between junctions that could be considered to be synaptic or nonsynaptic in the sense used by Dismukes.

Considering the location of synapses, there is also no hard and fast rule to be applied. The synapses about which we know most are those formed between the terminals of axons and the post junctional cell. These can be axosomatic or axodendritic in location. However, axoaxonic synapses have been demonstrated and are thought by many to be the basis of presynaptic inhibition. Why, therefore, should we be surprised when dendrodendritic synapses are demonstrated? Such synapses would merely serve to exemplify the diversity of neuronal interconnections. Provided that the prejunctional dendrite contains releasable transmitter, there is no reason why it should not release that transmitter in a voltage-dependent fashion; it remains to be shown that this does occur under physiological conditions. Whether that dendrite would release the transmitter in an all-or-none fashion might depend on whether it was capable of supporting propagated action potentials. Certainly some dendrites are capable of producing such action potentials (Llinas and Hess 1976).

The morphological differentiation of synaptic structures is also hard to define, with some showing more differentiation than others. I am, therefore, forced to the conclusion that the essential definition of a synapse must be based upon functional grounds, when two excitable elements communicate with each other by electrical or chemical means. A specified synapse can be further defined on an anatomical basis in relation to its differentiation of pre- and postsynaptic structures, synaptic cleft diameter, and neurotransmitter or transmitters but there is no single anatomical definition to encompass them all.

Is there any other factor that could impose a finite and invariable limit on the distance between release site and receptor site, which would prevent functional synaptic transmission? I think not. In any particular situation, the upper limit of separation for functional synaptic transmission will be imposed by the amount of transmitter released from the presynaptic element, the concentration required to produce a suprathreshold effect at the postjunctional site, the diffusion coefficient of the transmitter in the extracellular medium, and the rate at which the substance is removed from the medium by uptake and enzymic degradation as it diffuses through it. Clearly the maximum separation between pre- and postsynaptic elements could vary greatly from one situation to another, and until all of the operant factors are known, the limits cannot be determined.

There are two other aspects of synaptic transmission discussed by Dismukes that require some further comment. These are: the number of postsynaptic cells affected by one presynaptic element, and the speed and duration of transmission.

It is not necessary to limit the process of synaptic transmission to a one-to-one communication between excitable cells. It is the norm, rather than the exception, for divergence of lines of communication within the nervous system. The manner in which such divergence is achieved may well vary. It is accepted that a presynaptic axon branches in its terminal arborisations to contact many other neurones, thus achieving divergence. Divergence could also be achieved by liberation of transmitter in such amounts and at such distances from target cells that more than one could be affected by transmitter released from a single site.

There is no reason to suppose that such a mechanism, should it be demonstrated to exist, would inevitably lead to indiscriminate effects upon all neurones in the vicinity of the release site, regardless of physiological function. Whether a neurone in the vicinity would respond to the transmitter would depend not only on whether it had the appropriate receptors, but also on the precise geometrical relationship between the releasing and the receptive elements. Precise geometry is obvious in the cerebellum but is also present in cerebral cortex and is probably a feature of large parts of the nervous system, even when not so immediately obvious. Thus, even in the spinal cord, the Scheibels (1969; 1970) have demonstrated a precise geometrical arrangement of the elements, not only in laminae as described by Rexed, but in three-dimensional array. Hence we can imagine that some synapses are not associated with a clearly definable and small synaptic cleft but nevertheless manage to produce their effects in a functionally organised and specific fashion.

A common misconception about synaptic transmission is that it should occur rapidly and be of short duration. Fast and brief synaptic transmission may be expected in systems that analyse high-frequency components of the environment, as in the visual and auditory systems [see Wasserman & Kong: "Absolute Timing of Mental Activities" *BBS* 2(2) 1979]. Even here it may not be necessary to maintain the high-frequency response throughout the system. High speed and resolution of synaptic transmission may also be required in the execution of some fast reflex movements of the skeletal musculature. However, it must be obvious that other tonic controls of the musculature do not require high-speed resolution, and that there are many other bodily functions such as taste, smell, pain, and autonomic functions controlled by the nervous system that rarely if ever require high-speed resolution. The duration of synaptic events already observed by electrophysiological techniques range from the few milliseconds of postsynaptic potentials in motoneurones, to the hundreds of milliseconds duration of presynaptic inhibition, to the several second duration of the late discharge of Renshaw cells to antidromic stimulation of ventral roots. The slow onset of effects of putative neurotransmitters administered by microelectrophoresis cannot be taken as evidence that they must also produce slow effects when released from synapses; rate of onset is determined not only by the release charac-

teristics of the administering pipette, but also by the distance of the ejecting site from the recording site, which is usually close to the soma (Kelly 1975). In any event, a slow onset of effect is not necessarily incompatible with synaptic transmission, as will be evident from the preceding discussion. Nevertheless, it may imply a different mechanism from that involved in faster processes.

Convention is an ephemeral thing. Conventional mechanisms of excitation or inhibition in the 1950s and 1960s may have been increase in membrane conductance to specific ions. It is now clear that decreases in membrane conductance in response to transmitter action may also occur and may indeed in the end prove to be more common in the central nervous system: What was conventional yesterday, and is still so today, may become unconventional tomorrow! Other mechanisms of synaptic transmission might involve second messengers, as discussed by Dismukes, or even the modulation of the efficacy of activation of postsynaptic receptors by the transmitter, as I have recently proposed (Belcher and Ryall 1977) for the selective modification of the response to activation of the nicotinic receptors on Renshaw cells by substance P.

This brings me finally to the concept of modulation. I would agree with Dismukes that it is improper to differentiate between synaptic transmission and synaptic modulation. It is the function of synaptic transmission to modulate the activity of the postsynaptic element. This is true equally of inhibitory and excitatory inputs. We already have words in common physiological use that adequately describe effects of short- and long-term duration. These are the words phasic and tonic. Where the synaptic event elicited by the transmitter is of short duration, we could refer to phasic actions, and where they are of long duration, they could be called tonic. This qualification of the synaptic event has the merit of describing the character of the process, rather than limiting the meaning of the word modulate, which does not itself describe the event in any specific fashion.

In summary, I feel that there is no reason at the present time for postulating the existence of nonsynaptic (or nonjunctional) transmission, as distinct from neurohormonal effects exerted by substances transported to distant targest by the bloodstream or, possibly, via the corticospinal fluid. By avoiding the rather arbitrary division into synaptic and nonsynaptic transmission, we also avoid the problem of where within such a classification to place the autonomic neuro-effector junction. I also believe that there is no compelling reason to postulate that synaptic transmitters nonspecifically modulate the activity of vast arrays of functionally dissimilar neurones. Indeed, such a nonselective mechanism is incompatible, in my view, with what we know of the physiological organisation of the nervous system, in which control systems operate in selective fashions upon certain functional groups of neurones.

There is a case for qualitatively describing the morphological and functional differences between different synapses, but this is already happening. What is to me apparent is that the synapse can operate in a variety of morphological forms and by a variety of postsynaptic mechanisms, brought into play by a seemingly bewildering array of neurotransmitters, each with the possibility of interacting with more than one postsynaptic receptor. This merely illustrates the versatility of a nervous system that performs its complex and selective functions by mustering into organised units a multitude of diverse parameters.

by **Barry H. Smith**

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Polarity and modality of neuronal information transfer. Dr. Dismukes's paper addresses an important concept of neuronal function: that of multimodal information processing. The neuron has long been depicted as a computing unit in which there is a unipolar and unimodal information transfer system that begins with an algebraic summation of electrical input on dendrites and cell bodies and ends with the delivery by action potentials of essentially the same computed message to all axonal terminals. In this view, interneuronal communication is synaptic, being accomplished chemically by small molecules' producing postsynaptic electrical polarization changes that convey the transferred information.

We now know that the neuron is neither unipolar nor unimodal in its communication operations. For example, the neuron is not unipolar in that its input and output synapses are not necessarily segregated. In addition, it seems very likely that many, if not the majority, of central nervous system neurons use graded and not regenerative (action) potentials in intracellular communication (Schmitt, Dev, and Smith 1976). Finally, there is evidence, as reviewed by Dismukes, to suggest that interneuronal communication is not restricted to classical transmitter agents, and that, even with transmitters, the most important postsynaptic event is not always the production of a polarization change. For example, the regulatory metabolic effects of agents such as norepinephrine may in some cases be more important than the relatively short-lived electrical potential changes they produce, especially insofar as they may be amplified by systems such as those of the cyclic nucleotides (Bloom 1979). If there is nonsynaptic release of transmitter from nonsynaptic boutons of the "diffuse" adrenergic or serotonergic systems, then the relatively few neurons of these systems may release their transmitter contents into extracellular space to modulate the response of entire neuronal populations to more tightly coupled synaptic input that is concerned directly with the processing of on-line, externally-produced information.

The widespread occurrence and variety of synaptic and nonsynaptic molecular transfer in neuronal populations and the multiple regulatory effects exerted through such transfer suggest that it may be useful to think of chemical as well as electrical "circuitry" in the nervous system. Chemical "circuitry" seems particularly suited to achieve longer-lasting effects and "state" changes in single neurons as well as neuronal populations. Quite clearly, however, the electrical and chemical systems are interwoven, and it is in this interweaving that the unique information-handling and plastic properties of the neuron are achieved.

The multimodal aspects of neuronal communication have indeed generated terminological chaos. Whether the appealing simplicity of Dr. Dismukes's "neurohumor-neurohormone" classification will be sufficient to meet the demands of the systems (known now and yet to be described) remains an open question, as he himself points out. Not only additional terms but also different dimensions of agent classificaton (i.e. functional) may be required. One such dimension might be that of "effectors," "permittors," and "regulators" (Smith and Kreutzberg 1976).

Whatever the terminologies and classifications required, the new data and concepts of molecular communication among neurons have not simplified the problem of understanding the nervous system. At the most elementary level, the interweaving of the biochemical and electrical systems in a single neuron or a neuronal circuit requires new theoretical concepts to unravel the multiplicity of factors producing a given output. This output, furthermore, is not strictly predictable on the basis of the properties of the components producing it. At higher organizational levels, the problems seem more formidable and yet are already beginning to yield to quantitative theoretical approaches (for a review, see Reichardt and Poggio 1979). Recognition that system properties may not be predictable on the basis of component parameters, and that both "top-down" and "bottom-up" approaches are needed for a successful attack on nervous system function, are significant steps forward. Meanwhile, the challenge of understanding nervous system functional organization has become all the more fascinating.

by **T. Tömböl***

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Modulation and neurotransmitters. It is not accidental that Dismukes, in dealing with new concepts of molecular communication among neurons, discusses the notion of modulation. In the words of his review, the term modulation "is increasingly being used to characterize the action of putative transmitters" in diverse contexts; further on, the term is applied at the circuit and behavioral level.

In his analysis of the concept of modulation Dismukes argues pro and contra, using both his own explicit definitions and more informal concepts at various levels. His arguments are undoubtedly pertinent,

and evaluating the neurotransmitter and neuromodulator concept is extremely appropriate in attempts to characterize the central and peripheral effects of norepinephrine. He concludes that our knowledge is not yet sufficient for an exact definition of the concept and that it is therefore difficult to determine clearly what should be termed "modulator action." He finally suggests applying the concept of modulation in a wide and general sense rather than reserving it for a special function. It is, according to Dismukes, not appropriate to restrict the concept of a neuroregulator or neuromodulator to a particular transmitter; rather, it should be regarded as a special system enabling a given agent to induce different responses in different regions.

Although I am basically in agreement with these arguments and conclusions, it seems to me that while using the concept in the general sense one could still narrow its meaning somewhat, even in view of our current knowledge. In principle the domain of modulation is unambiguous: it somehow promotes and effects adaptation to the particular circumstances involved in the performance of a function. Various levels may be involved in performing any function, and at all levels modulation may take place according to structural and molecular specifities.

I shall attempt to illustrate the above points with concrete examples: At the subcortical station of the ascending pathways in the thalamus, an examination of the structure of the VPL, CGL or CGM nuclei reveals that different structural elements are involved in conveying information. Two types of interneurons modifying the function (i.e. transmission) of the relay neurons can be found at this level. The fibers (cortical and reticular) arriving from different loci also influence information transmission in the thalamic nuclei. Via their connections they may express their effects through both the relay neurons and the interneurons.

As regards the thalamus, the number of interneuron types varies in different species. In lower species only one interneuron type can be found in the thalamic nuclei. Quantitative measurements to date reveal that the proportion of relay- and interneurons varies too: the number of interneurons in the cat exceeds that found in the rat or rabbit. This observation refers to the fact that in higher species more possibilities and variations offer themselves for modulation of afferent impulses at the thalamic level.

In the cortex the number of interneuron types is several times greater than in subcortical regions and a difference may also be observed in the phylogenetic sequence. For this reason it can be inferred that the structural elements involved in the modulation play a fundamental role in the elaboration of the stimulus [see also Steriade: "Cortical Long-Axoned Cells and Putative Interneurons in the Sleep-Waking Cycle" *BBS* 1(3) 1978].

The effect of the transmitters implicitly involved in the modulation cannot be evaluated independently of the neuronal structural elements from whose axon terminals they are released. It might thus be possible that the transmitter released from the axon terminals of the interneuron has a modulator effect there, although it may be identical with the transmitter released from the terminal of the relay-neurons. At the molecular level (considered exclusively morphologically) vesicles of two different types can be observed in one and the same terminal. In the mossy fibers of the hippocampus, for instance, aside from the number of light vesicles, vesicles with large granules are also observable in not insignificant numbers in the terminals. It could be postulated that the vesicles with large granules modify the release of the transmitter-substance from the light vesicles.

One could go on enumerating examples of possibilities of modulation – and conclude by raising the problem of membrane receptors. As a matter of fact, the phenomenon of modulation cannot be examined by picking it out and isolating it from the whole system in which, as a partial phenomenon, it becomes manifest in some region and at some level. On the other hand, it is of outstanding importance, since after all, the phenomenon of adaptation ensues from the modulating capability of the nervous system.

[°]Received too late for Response from author.

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Neurohypophyseal hormone release. The problem raised by R. Key Dismukes can be simply summarized in two questions. The first: Can Dale's Principle be regarded as a theory demonstrated by experimental data? The second: How does one classify various compounds, consisting of small or large molecules with varying ease of activation, synthesized, taken up, stored, and released by neurons?

At present, much evidence accumulated indictating that neurons communicate by the mechanism of exocytosis of stored compounds. It is unlikely that compounds classified as neurotransmitters or neuromodulators or otherwise can be stored in chemically pure form. It is more likely that they are stored on a carrier or carriers, and released with them. It is also improbable that the carrier released in the synaptic cleft would be neutral with respect to the postsynaptic receptors and enzymes inactivating the transmitter compounds. The separation of the active compounds from the carrier could also occur at different rates.

The release of neurohypophyseal hormones from endings of neurosecretory neurons and their carrier-proteins, neurophysins, occurs simultaneously. This mechanism cannot be regarded as an exception in neuronal communication. The posterior pituitary lobe, with its neurosecretory neuronal endings, is a burden for neuroscientists, but it cannot be regarded as an exception.

There is a longstanding tendency to attempt to classify all physiological processes occuring in the organism. We find this instructive. I agree with the definitions of neurohumor, synaptic neurotransmitter, nonsynaptic transmitter, and neurohormone, but clear, distinct definitions for neurotransmitter and neuromodulator can not be easily formulated. In my textbook for medical students small molecular weight compounds such as acetylcholine, amines, and amino acids are classified as neurotransmitters, while neuropeptides are classified as neuromodulators; my impression, however, is that this is a rather superficial classification. It ought to be applied according to the results of a standardized testing procedure on some well-identified cells under resting and specific excited conditions. The reinvestigation of all putative neurotransmitters and neuromodulators under standard conditions should allow a better definition of what we call neuromodulator and neurotransmitter.

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Hormones as modulators of neuronal activity. The review by Dismukes admirably pulls together some intriguing aspects of "nonsynaptic" communication between nerve cells. The data, coming from widely scattered disciplines within neurobiology, are only gradually beginning to fit into a cohesive framework. Midway through the article, Dismukes states that at this point there is little advantage in attempting to distinguish modulators from transmitters, but later in the paper he succumbs to temptation and puts forth a scheme of classification.

I disagree with a number of aspects of Dismukes's proposed nomenclature. The generic term "neurohumor" is archaic and has fallen out of general usage. I do not see an advantage in bringing it back. I agree with retaining the classical delineation of neurotransmitter substances that are secreted into tissue spaces, and neurohormones (or neurosecretory hormones) that are released into the blood or cerebrospinal fluid. However, there seems little value in subdividing the transmitters based on the type of junction from which they are released. This division does not carry a clear functional distinction, and some molecules would be placed in either category on a case-by-case basis. It would seem better that the action of the substance, classical transmission as opposed to modulation, and the anatomy of the terminal should be treated separately from the classification of the released product. Thus, one would talk about the modulatory action of a transmitter (or a neurohormone) rather than calling the substance itself a neuromodulator or some other such term.

It is also worth pointing out that this new mode of communication is not unique to nerve cells. This communication has its roots in endocrinology, and interactions between endocrine cells and neurons show features that are pertinent to many topics in this review. Peptides such as angiotensin, ACTH, and prolactin have nonneural origins but can have behavioral actions like the neurohormones. Also, steroid hormones, which do not have counterparts in the nervous system, act on specific target sites within the CNS and cause long-latency,

long-duration alterations in neuronal function (McEwen et al. 1979).

As pointed out by Dismukes, one of the intriguing aspects of neural modulation is that complex information-processing can ocur at the level of individual neurons. This processing undoubtedly involves changes in the biochemical machinery of the target nerve cell. The heterogeneous nature of the nervous system severely complicates a biochemical analysis of the changes evoked during this modulation. Hormones that modulate nervous activity also act on nonneural tissues. Thus, their biochemical action on target cells can be more readily studied in a relatively homogeneous tissue. This information can then be used as a guide in designing experiments to link the biochemical changes in neurons with alterations in their membrane physiology. The early work by Sutherland and collaborators, showing that epinephrine acted via cyclic AMP (Sutherland 1972), provided not only the groundwork for elucidating the action of certain peptide and amine hormones, but also a valuable model for the study of peptide and amine action in the nervous system (Kupfermann 1979). Similarly, the current models for the genomic action of steroid hormones (Gorski and Gannon 1976; O'Malley and Means 1976) will probably apply to many of the modulatory actions of these compounds in the CNS and should help in the elucidation of the means by which nuclear activity results in new functional states in these cells.

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Communication at synapses. In recent years, the recognition of the importance of the synapse as the primary site for the processing of information between neurons (see Weight 1979), together with the growth of research in the neurosciences, has resulted in a proliferation of data on the morphology, chemistry, pharmacology, and physiology of synapses. The plethora of information and the varied nature of the data, however, result in a potpourri of observations. Dismukes touches upon a number of interesting new aspects of molecular communication between neurons, intending his paper as a springboard for discussion of neuronal information-processing. The numerous topics covered reflect the great diversity of information in this rapidly developing area of neuroscience; however, the diversity of information also makes it difficult to discern clearcut trends. I will briefly attempt to delineate a few trends that I see emerging in our understanding of communication at synapses.

Long-lasting synaptic potentials. One of the recent trends in our understanding of synaptic communication is the growing recognition that a synaptic potential can have a long-lasting time course. Traditionally, synaptic potentials have been considered to be very brief events lasting only a few, or at most, tens of milliseconds. This is due to the fact that the majority of synaptic junctions investigated in detail have been in sensory or motor systems, where the postsynaptic potentials (PSPs) have time courses in the millisecond range (see Eccles 1964). In the 1960s, however, investigations using the iontophoretic technique to apply putative neurotransmitters to neurons in the central nervous system (CNS) revealed that transmitters such as norepinephrine (NE), dopamine (DA), serotonin (5-HT), and acetylcholine (ACh) often produce responses lasting from many seconds to many minutes following termination of the application. These responses contrast to the very brief actions of amino acids such as GABA, glycine, and glutamate. During the same time period, histochemical techniques were developed that provided evidence for synaptic pathways containing, NE, DA, 5-HT, and ACh in the mammalian CNS. Those pathways were not a part of major sensory or motor systems, but rather they arose in regions such as the brain stem for ACh, the locus coeruleus for NE, the substantia nigra for DA, and the raphe nuclei for 5-HT. The distribution of those pathways was widespread throughout the CNS, and the function of the pathways was essentially unknown. In addition, such pathways were found to be difficult to investigate with electrophysiological methods because of technical problems due to stimulus spread to adjacent low-threshold axons or to the activation of fibers passing through the region when electrical stimuli were applied.

Subsequent investigations on sympathetic ganglia revealed that synaptic potentials lasting many seconds to minutes can be elicited by the selective stimulation of preganglionic pathways (for review see Weight 1974a). In addition, the brief administration of the neurotransmitters also resulted in the generation of long-duration postsynaptic potentials in sympathetic neurons. The experiments on sympathetic ganglia obviated many of the technical problems encountered in the CNS, such as stimulus spread and the possibility of activating polysynaptic pathways or reverberating circuits, and they clearly demonstrated that a PSP can indeed have a long-lasting time course. Recently, experiments on the CNS have suggested that stimulation of central monoaminergic and cholinergic pathways can result in long-lasting postsynaptic responses that appear to be due to the activation of pathways mediated by these neurotransmitters. In view of these data, I do not find it particularly surprising that, in some cases, monoamine transmitters are located in axonal structures lacking synaptic contact. As discussed above, several lines of evidence indicate that monoamine pathways are frequently associated with the generation of long-lasting or slow postsynaptic responses. Thus, at such synapses there is no need for synaptic specialization to provide the rapid transfer of information. Moreover, the morphology of possible monoamine release sites in the CNS is similar, in many respects, to the morphology at peripheral adrenergic junctions – e.g., the junction of postganglionic sympathetic axons with smooth muscle. In this regard, it is interesting to note that NE also produces a long-lasting response in smooth muscle.

A few words should also be said about CNS responses with extremely long time courses. Experiments on amphibian sympathetic ganglia indicate that there is a postsynaptic potential, designated the "late-slow EPSP," that has a time course lasting up to 20 minutes (e.g., see Schulman and Weight 1976). In a recent report Jan et al. (1979) provided evidence that the transmitter mediating the late-slow EPSP may be the peptide LHRH (luteinizing hormone-releasing hormone). We have found evidence that this very long-lasting PSP is capable of modulating (enhancing) synaptic transmission in another synaptic pathway (see Schulman and Weight 1976). By all of the criteria that we have examined, the late-slow EPSP is a bona fide synaptic potential. In view of the fact that the postsynaptic action of this long-lasting peptide neurotransmitter can modulate transmission in another synaptic pathway, I do not believe that our present state of knowledge is sufficient to classify various long-lasting responses into categories such as neurohumoral, neurohormonal, neuromodulatory, or neuroregulatory. Such classification may lead to disagreements over definitions and semantics, and in doing so it could obscure the more important issue of the functional significance of such responses (see below).

Intracellular mediators and transmitter action. A second trend in our understanding of communication at synapses is the development of the concept that an intracellular substance or "second messenger" may mediate the postsynaptic action of a neurotransmitter at synaptic junctions. In the electrogenesis of fast PSPs, such as the endplate potential at the neuromuscular junction, there appears to be a close physicochemical coupling between the activation of the postsynaptic receptor and the permeability change of the postsynaptic membrane. However, the duration of slow PSPs, as discussed above, is considerably longer than the presumed duration of receptor activation. Moreover, the distribution of postsynaptic receptors is usually limited to the subsynaptic region, but the permeability change that generates slow PSPs appears to involve most of the neuronal membrane (see Weight et al. 1979). Such considerations have led to the idea that the action of the neurotransmitter on postsynaptic receptors results in a change in an intracellular substance, which in turn leads to the membrane permeability change that generates the postsynaptic potential – viz., the intracellular substance acts as a second messenger in mediating the activation of the receptor.

Cyclic nucleotides have been proposed as possible intracellular second messengers mediating the generation of postsynaptic potentials in neurons. This hypothesis is based, in part, upon data from experiments on sympathetic ganglia (for review see Greegard 1976). However, recent experiments in several laboratories, including our own, have been unable to substantiate a number of the observations upon which this hypothesis is based (for review see Weight et al. 1979). For example, in sympathetic neurons it has not been possible to

demonstrate that cyclic AMP or cyclic GMP can elicit the membrane permeability changes involved in PSP generation. Furthermore, drugs that affect cyclic nucleotide metabolism do not affect appropriately the postsynpatic potentials presumed to be mediated by cyclic neucleotides. On the basis of these recent studies, it would appear that cyclic nucleotides are probably not the intracellular second messengers mediating the generation of the PSPs. Nevertheless, neurotransmitter activation of postsynaptic receptors does increase the cyclic nucleotide content. This suggests that PSP generation and cyclic nucleotide elevation may be parallel results of postsynaptic receptor activation. It is possible that cyclic nucleotides may function as intracellular second messengers, but the message conveyed and the physiological effects of their action still remain to be determined. Additionally, the generation of the slow postsynaptic potentials presumably involves the mediation of an intracellular second messenger, but the nature of the second message is at present unknown.

Intracellular calcium and membrane permeability. A third area of recently-developing concepts is the role of calcium in controlling neuronal membrane permeability. Following the demonstration by Meech that the intracellular injection of calcium ions increases the potassium permeability of neuronal membrane, a number of studies have investigated the role of calcium in controlling neuronal membrane permeability (for review see Meech 1978). Voltage-clamp studies have shown that the entry of calcium ions into several types of neurons activates an increase in membrane potassium permeability, and this calcium-dependent potassium activation appears to have important functional consequences for the physiology of neurons. The spike-firing frequency of a neuron is limited by the duration of the absolute and the relative refractory periods following an action potential. The refractory periods are a function of the spike afterpotentials. In several types of neurons the afterhyperpolarization following an action potential consists of two components: a voltage-sensitive potassium conductance and a calcium-sensitive potassium conductance (Barrett and Barrett 1976; Busis and Weight 1976). The calcium-sensitive component is due to the entry of calcium ions during the action potential. Furthermore, it has been found that the calcium-sensitive potassium conductance is prolonged in duration by theophylline (Busis and Weight 1976). Recent studies indicate that the potentiation of this conductance change by theophylline is independent of cyclic nucleotide elevation (Smith, Weight, and Lehne 1979). Since the refractory period of a neuron is related to the afterpotential, the increased duration of the spike AH produced by theophylline prolongs the duration of the relative refractory period. Thus, the control of this calcium-sensitive membrane-permeability change is important in the regulation of neuronal excitability and the frequency of spike-firing.

Generation of synaptic potentials by closing membrane ion channels. A fourth area of developing information is the accumulation of data indicating a widespread distribution of synaptic potentials generated by the closing of membrane ion channels. Previous investigations on fast synaptic potentials, such as the endplate potential at the neuromuscular junction, had demonstrated that they are generated by increasing membrane permeability to certain ions (see Eccles 1964). Subsequently, studies on the long-lasting or slow PSPs in sympathetic ganglia revealed that synaptic potentials can also be generated by decreasing membrane permeability (i.e. closing membrane ion channels; see Weight 1974b). Initially, the generation of postsynaptic potentials by this mechanism was felt to be unconventional and perhaps an uncommon event in the nervous system. More recent investigations, however, reveal that PSPs generated by decreased membrane permeability have a widespread distribution throughout the nervous system of both vertebrates and invertebrates (for review and references see Weight et al. 1979). In the vertebrate nervous system it has been reported that such mechanisms are involved in ACh responses in neocortex, hippocampus, and spinal cord, NE responses in cerebellum and spinal cord, and 5-HT and substance P responses of Auerbach's plexus neurons. For the invertebrate nervous system, both EPSPs and IPSPs in several different types of neurons have been reported to be due to conductance-decrease mechanisms, as have responses to several putative neurotransmitters such as 5-HT, ACh, histamine, and GABA. In addition, most of the postsynaptic responses generated by decreased membrane conductance have a prolonged time course. The widespread distribution of these responses, together with their long-lasting time course, suggests that the closing of membrane ion channels may be a general mechanism in the nervous system for the generation of long-lasting synaptic potentials.

Long-lasting modulation of synaptic excitability and learning. The final trend that I will briefly discuss is the long-lasting modulation of synaptic excitability. Little is known regarding the cellular mechanisms that underlie learning, although many neurobiologists believe that functional changes in the properties of nerve cells or modification of synaptic mechanisms may underlie much of the plasticity in the nervous system. One cellular hypothesis for learning, suggested several years ago, proposed that the repeated use of one synaptic pathway may produce a postsynaptic change that would result in a lasting enhancement in the efficacy of synaptic transmission in a second pathway. In view of this possibility, it is important to determine experimentally whether the stimulation of one synaptic pathway can result in postsynaptic changes that will alter the effectiveness of transmission in another synaptic pathway.

A number of studies have demonstrated alterations of synaptic transmission produced by presynaptic mechanisms (see Martin 1977). Until recently, however, there has been little evidence for enhancement of synaptic transmission by postsynaptic mechanisms. As discussed above, studies on sympathetic neurons have revealed synaptic potentials generated by decreases in membrane permeability. The decreased permeability is manifested electrophysiologically as an increase in membrane resistance. On the basis of Ohm's Law, it was predicted that the increase of membrane resistance would result in an increase in the amplitude of fast EPSPs. This prediction has been verified by the observation that fast EPSPs can be increased in both amplitude and duration during a synaptically-induced decrease in membrane permeability. Moreover, the augmented fast EPSP amplitude results in an increased efficacy of impulse transmission across the synapse (Schulman and Weight 1976). The enhancement of synaptic efficacy is also long-lasting by virtue of the long duration of the decreased-conductance synaptic potential.

Other recent reports also indicate an enhancement of neuronal excitability associated with decreased membrane conductance: in *Aplysia* a decreased conductance PSP has been found to be associated with the augmentation of a behavioral reflex (Carew and Kandel 1977); in cortical pyramidal neurons an increased membrane excitability has been reported to be associated with a learning paradigm (Woody and Black-Cleworth 1973); and membrane resistance is increased in locust motoneurons trained by a computer to fire at higher frequencies (Woollacott and Hoyle 1976). Clearly, more work is needed to clarify the role of decreased-conductance mechanisms in these phenomena. Nevertheless, such studies point to the possibility that the regulation of postsynaptic membrane permeability may be a fruitful area for future investigations on the long-term control of synaptic excitability in neurons.

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Restricted extracellular pathways for molecular communication?

Dismukes presents a useful review of possible new kinds of neuronal communication. Three further examples of potential molecular communication among neurons would be the nonvesicular (nonquantal) release of transmitter from neurons, the release of proteins by brain cells into cerebrospinal fluid, and the release of rapidly-transported proteins from nerves. These are briefly described below.

The amount of nonvesicular release of transmitter from rodent and frog motoneurons has been reported to be many times the amount of resting, quantally-released transmitter (Mitchell and Silver 1963; Vizi and Vyskocil 1979). Such nonquantally-released transmitter has effects on the resting potential of neighboring cells (Katz and Miledi 1977).

The metabolism of some of the proteins in cerebrospinal fluid appears to be related to behavioral experiences (Shashoua 1977; Benowitz and Shashoua 1977). While the source of these proteins is

nonneuronal, the triggering of the enhanced synthesis, and the target for the proteins, could well be neuronal.

A recent report by Hines and Garwood (1977), while clearly preliminary, suggests that some rapidly-transported neuronal proteins could be secreted from axons during transport. Should this source for cell-to-cell molecular communication be established, the targets for the molecules would be important to identify.

The numerous examples cited by Dismukes, and the three additional examples described above, all point to the possible importance of the structuring of extracellular pathways within the central nervous system. If there are nonsynaptic transmitters and neurohormones, there may also be restricted pathways and channels for them within the central nervous system. In this way neurohumors released from one neuron would reach only selected targets. Other cells with similar receptors, even nearby ones, would not be affected if extracellular barriers were imposed. A selective circuitry for nonsynaptic transmitters, entirely separate from the normally considered electrical and synaptic circuits, could thereby be established in the brain.

As Dismukes indicated, we may be entering an exciting new phase of understanding of neuronal communication. The exploration of extracellular pathways for modulator molecules could become of key importance to that understanding.

Finally, concerning the definitions that Dismukes offers for classifying neurohumors, one may ultimately wish to distinguish the agents on the basis of their actions on target cells. For instance, one could class the agents according to whether their primary influence is on transport and conduction properties, enzymatic activities, or gene expression in the target cells. But perhaps all such definitions should await firmer data on mechanisms.

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Re-evaluation of norepinephrine function: a potential neuromodulatory role? Dismukes's target article captures well the current thinking about molecular communication among neurons. We empathize with the struggle of the author concerning the weak nomenclature and terminology in use in the area. Considerable academic energy has been exerted at meetings, in papers, or in reviews, simply to define the meaning of terms. Regretably, the author's peculiar problem in this article is that he stumbles over his own awareness. He comes to realize that a precise definition of "modulation" is not yet possible, yet he cannot avoid the temptation to formulate one of his own.

"Modulation" has become most useful as a linguistic junk bag into which we put processes we don't understand. Such processes defy our attempts to classify their actions or mode of operation according to extant conventional criteria (Werman 1966). It is something like the concept of a "trophic" substance. When the characteristics of a particular form of molecular communication become thoroughly defined experimentally, one is entitled to invent a new word or phrase. Still, there is considerable ambivalence to our current situation, because we would like to enjoy the use of a precise terminology well before we have done the experimental analysis to earn it.

Our suspicion is that progress in the characterization of central "synaptic transmitters" has been somewhat hindered by the establishment of rather precise "experimental criteria" for their identification. The defining of "criteria" evolved from a concerted effort over several decades, which clarified the role of acetylcholine as a transmitter at the neuromuscular junction. The result of establishing such "criteria" had the unexpected adverse effect of making one feel that a substance wasn't a "transmitter" unless it acted like acetylcholine at the neuromuscular junction, or worse, that something was inherently wrong with the experimental results. If there is truth to this suspicion, it might account for the resistance of many scientists in the early 1970's to accepting the concept that substances released from neurons could do other than increase conductance channels.

Expectations as to how transmitters or modulators are supposed to work can lead to distorted interpretations of the rapidly expanding literature. A prime example is provided by Dismukes's article, in which the ability of norepinephrine (NE) iontophoresis to enhance conventional synaptic actions in cerebellum (Freedman et al. 1977) was attributed to the reputed "increase in resistance of the Purkinje cell membrane" produced by NE. The discussion in the cited work states the contrary: that the results were quite difficult to interpret on that basis alone. We originally thought that the fall in conductance would be obligatorily linked to whatever caused the depression of spontaneous neuronal firing. The unexpected finding in that initial study was that such changes in the efficacy of the synaptic inputs could be observed at concentrations of NE having no direct inhibitory effects on Purkinje cell discharge, as well as at times long after recovery of baseline firing rates from the depressant actions of NE. The question raised in that discussion was how a change in membrane resistance could selectively enhance synaptically-evoked discharge, without exerting simultaneous effects on background firing. Although possible, it is not what one might expect.

Our concern in this laboratory has been with the creation of a fruitful experimental strategy from which definitions of modulatory actions of norepinephrine will naturally emerge. Following initial studies of interactions between NE and conventional synaptic inputs in cerebellum (Freedman et al. 1977), further studies have been conducted to examine the influence of NE on Purkinje neuron responses to iontophoretically-applied putative transmitter substances (Moises et al. 1979). In addition, endogenous NE, released via stimulation of the locus coeruleus, interacts with neuronal responses to iontophoretically- and synaptically-released transmitter substances (Moises and Woodward 1979; Moises et al. 1978). These cerebellar studies have been followed by a similar series of experiments in cerebral cortex to generalize results to other areas of the brain receiving noradrenergic innervation (Waterhouse and Woodward 1979; Waterhouse et al. 1978). The above interactions have also been tested after destruction of NE terminals by 6-OH-dopamine (Moises 1979). In addition, many other drugs have been tested to examine specificity of the noradrenergic "modulatory" effects on transsynaptically and putative transmitter-evoked neuronal activity. Each of the experimental results has contributed to the definition of modulation as it applies to NE (Woodward et al. 1979). In summary, we don't believe that adequate definitions of "neuromodulation" can be constructed from common parlance, but rather that they will evolve from careful assessment of experimental results.

Our current hypothesis is that postsynaptic processes that mediate conventional transmitter efficacy may be regulated by NE. A direct enhancement of postsynaptic receptor action is a strong possibility, but a slowing of the kinetics of transmitter inactivation is also to be considered in future studies.

While we worry frequently about the mechanisms of action of putative "modulators," we often fail to be sufficiently concerned about the basic determination of their physiological role. Acetylcholine at the neuromuscular junction is peculiar in that its physiologic purpose is clearly to cause muscles to twitch. Furthermore, little imagination is required to ascribe simple synaptic functions to conventional excitatory or inhibitory agents in the central nervous system. In contrast, no simple elementary role in synaptic integration can be ascribed to NE, or to many other chemical substances, for that matter. Too often our strategies rely on a faith that a reductionist approach for getting at biophysical mechanisms will lead most quickly to a global understanding of the physiology of how things work.

We feel that the highest priority should be placed on demonstrating which elemental phenomenon (such as depression of activity or enhancement of synaptic effectiveness) mediates the global function of NE and how. Such a gross teleological analysis may be desperately needed to save us from sterile researches on the doubtful significance of trendy epiphenomena, which may be the real danger in current research on "modulatory" substances.

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A note of caution in neurohumor nomenclature. It is important not to jump too quickly to conclusions based on the localization of various

neuropeptides in the mammalian brain. This comment is especially aimed at studies undertaken with immunohistochemistry, in which the specificity of the antigens has not been clearly demonstrated. The lack of specificity is often apparent in the high levels of background-staining or fluorescence in various brain areas. While this technique is new, exciting, and offers a potential major advance in the localization of peptide neurotransmitters, it would also appear to be beset with artifact unless very highly purified antigens can be utilized. It may thus be premature to be considering neurotransmitter actions for some of the hypothalamic peptides just because they are "found" in other parts of the brain. Until the localization of a particular peptide can be conclusively proven, one must view with caution all the subsequent claims as to its role as a neurotransmitter based solely upon its having an action on nerve cells.

Furthermore, since most peptides will most probably do something to most nerve cells just by the nature of charge distribution inherent in the molecule (i.e. electronegative carboxyl groups and electropositive amine groups), no precise actions are likely to be elaborated until specific blockers for the peptides can be found.

The problem raised by these studies involves how one shall ever be able to follow the literature, which is rapidly becoming totally confused by preliminary reports and premature findings that were done hurriedly, if not sloppily, with the antigen that seemed to work best at the time but clearly did not lead to definitive binding of only the specific peptide under study.

In the case of angiotensin II, which has been demonstrated to be "localized" in brain tissue, it is not clear if it may have crossed the blood-brain barrier to enter the brain or is also synthesized within the brain.

Neurohormones versus neurotransmitters. The elegant work of Victoria Chan-Palay (1976), in which serotonin-containing axons and terminals form very concentrated networks surrounding the aqueducts, actually extending into the ventricles of the brain, is certainly most suggestive of a neurohormonal role of 5-hydroxytryptamine (5HT). The question of what 5HT does upon release into the cerebrospinal fluid (CSF) will continue to intrigue us. Its role in producing sleep is now quite well-defined, and it may be that activation of raphe units causing a widespread hormonal release of 5HT delivered through the ventricular system will be an important underlying mechanism involved in the etiology of sleep. It may also be of consequence in the regulation of vascular tone to surface blood vessels of the brain that are bathed constantly with CSF. Thus in the case of 5HT and its vastly complex arborized network of fibers innervating the aqueductal areas of the ventricular system, it may be possible in the near future to accurately describe some of the actions of a "nonsynaptic transmitter" – or is it a "neurohormone" type of neurohumor?

A different class of substance action that could hopefully be embodied in the proposed scheme of classification concerns the actions of ions. In the vicinity of the afferent terminals of the spinal cord, K^+ ion has been observed to increase extracellularly following afferent activation. Since K^+ ion is used by a large number of investigators to demonstrate neurotransmitter release *in vitro* as well as *in vivo,* the question arises as to whether K^+ ion accumulation with ongoing synaptic activity may also be a physiological mechanism to regulate the amount of depolarization of afferent terminals within a localized mini-environment, and may in effect also be contributing to such mechanisms as presynaptic inhibition, and so forth.

Similarly, the role of Ca^{++} ion is currently being assessed in a variety of cell-to-cell interactions. In particular, the phenomenon of electrical coupling between cells is quite widespread among nonneural cells, forming various epithelial and organ tissues of the body. In these cells Ca^{++} ion concentration seems to be crucial in determining whether electrical coupling or uncoupling will occur. In the mammalian central nervous system, evidence for coupling between dendrites exists in several distinct identifiable types of neurons and may explain some of the bursting patterns of discharge of these cells. Is the local Ca^{++} ion concentration also important in regulating such coupling, and if so, under what class of neurohumor will it be identified? The close, intimate apposition of neural elements characteristic of some coupled junctions may also be a consequence of their local tissue water balance, achieved through osmotic regulation by cellular metabolism. Such close contact may also form the basis for the burst type of epileptiform discharge observed in epilepsy. It is of interest that a drug such as hydantoin, which is known to cause cessation of such discharge, is also noted to cause marked shifts in fluid compartments, perhaps resulting in a separation of the neural elements responsible for coupled bursting. Perhaps these neurons only achieved their epileptiform properties when closely apposing one another. The question is: can you label *water* as a neurohormone, or Ca^{++}?

The problems raised by these types of interactions, in which simple ionic constituents in the microenvironment of the cell may regulate changes in cell or terminal excitability in much the same way proposed for adenosine, and the question of whether they should be termed neurohumors, cannot be clearly resolved at present. Perhaps caution should be applied to prevent our zealous placing of labels on substances whose actions are not completely understood at present.

Author's Response

by **R. Key Dismukes**

Discussing new neurocommunication concepts: complements, counterdefinitions and counterexamples

Categories of neuroactive substances

ARCH & PALAY suggest that we should not concern ourselves overly with terminology, and that varied, sometimes conflicting uses of terms such as "modulator" and "transmitter" simply reflect the incompleteness of the data about which we speculate. Indeed, it would be silly to try to make our nomenclature completely tidy, and I strongly agree with BARKER, and with LEMBECK who point out that nomenclature must be derived from experimental observations rather than preconceived categories into which data is forced, Procrustean fashion. However, most commentators found it important to discuss terminology, for reasons that I would summarize as follows:

1. Words that are used to characterize or categorize neuroactive substances (e.g. transmitter, modulator, regulator) and their actions often appear to be straightforward and clarifying when used without explicit definition or reference to physiological mechanisms. This appealing simplicity, however, can disguise considerable looseness of thinking. For example, as WOODWARD, MOISES, & WATERHOUSE and IVERSEN point out, "modulation" has become a kind of linguistic junk bag for processes we don't understand.

2. Categorizations that are implicit rather than explicitly stated can perpetuate mind-sets that cloud our sensitivity to unexpected features of neuronal action (the unwitting biases described by ELLIOTT & BARCHAS; see also LEMBECK).

PALAY presents an appealing picture of the open-mindedness of neuroanatomists and neurophysiologists, suggesting that it is only "neuron modelers and information specialists" who have rigid concepts of the unitary nerve cell. While I would like to share his benign view of experimentalists, I wonder if it is entirely supported by the experiences of those physiologists and anatomists presenting evidence for nonconventional functions (see, for example, the discussion of Woodward et al.). At any rate, it is clear that our concepts must be sharply framed if there is to be a useful interplay between experiment and theory. My purpose in this section of the paper was not to quibble over semantics, but to tease out assumptions underlying the categories to which we assign neuroactive substances.

Several commentators (e.g. TRUMAN) found the "neurohumor" category archaic and unnecessary. I included this term as a supercategory because the range of forms and actions of both neurotransmitters and neurohormones is broad and there appear to be grey areas

that will be hard to categorize. Having such a term allows us to refer to a neuroactive substance (HOYLE's term) without being overly restrictive when we are not yet clear as to the exact nature of its function (a not uncommon situation!).

I had assumed that my discussions implicitly ruled out small ions, such as K+, as neurohumors, but since FLOREY and PHILLIS raise the point, I should amend the definition to be more explicit; thus, let *neurohumor* be a generic term for a substance released by a neuron to alter the activity of other cells, adjacent or distant. Various mechanisms of action may be employed, but in general neurohumors operate through specific receptors that mediate the cellular response(s).

I deliberately exclude from the neurohumor category substances of nonneuronal origin, even though ELIOTT & BARCHAS argue for inclusion. Certainly, substances such as adrenal steroids and glucose play important roles in regulating neuronal activity, but I feel that they do not fit within generally held concepts of neuronal transmission (see DUNN's commentary), and that it is worthwhile to distinguish modulatory agents of neuronal and nonneuronal origin. HATTON and PHILLIS remind us that glial cells may modulate neuronal activity, and their discussions of control of extracellular K+ concentration are a valuable addition to concepts of regulation.

PHILLIS is quite right in pointing out that I make no distinction between the *actions* of junctional (or synaptic) neurotransmitters and nonjunctional neurotransmitters. As PALAY emphasizes, there is no great difference between the local action of a transmitter at a synapse and its more diffuse action in the absence of synapses. However, as I pointed out in my target article, the kinds of information conveyed by the two forms of transmission may differ considerably. I am a little nervous about the distinction I made (transmission to one target cell versus transmission to multiple cells), because I do not want to imply that there are only two forms of transmission. Rather, there may exist a spectrum of transmission modes, ranging from morphologically specialized synapses between contiguous cells to diffusion to distant multiple targets. Sharp lines of demarcation may not be found.

RYALL makes the intriguing suggestion that the term synapse be used to characterize the functional interaction between excitable cells, without specifying morphological dimensions. Although this has appealing logical consistency, I think it would be hard to divest the term of the morphological connotations that have grown since Sherrington's time. I find it hard to think of the great space between releasing site and multiple targets suggested by Chan-Palay (*oper. cit.*) for serotonin release into the ventricular system as a "synapse." LIBET suggests an intermediate approach in which the term "loose synaptic" be used instead of "nonsynaptic" so that the functional connotation would be retained.

The distinction between neurotransmitters and neurohormones is much more clear cut. Neurotransmitters, directly or indirectly, alter the electrical responsiveness of excitable cells (this includes modulation of postsynaptic responses to other inputs and modulation of presynaptic depolarization-release coupling). Neurohormones produce actions that do not primarily affect electrical responsiveness. Since FLOREY takes exception to my wording, I will attempt clarification by rewording the definition as follows: *neurohormone:* a neurohumor capable of regulating multiple target cells, often distant and of diverse types. The primary response evoked is not a change in electrical responsiveness; in fact, the target cells are often not electrically excitable. Transport is generally via vascular channels.

DUNN's proposal to dispense with the criterion of distance altogether and distinguish neurotransmitters from neurohormones solely on the basis of alteration of membrane potential has appealing simplicity and straightforwardness, but it would encounter problems. LIBET describes a system in which dopamine modulates evoked postsynaptic potentials without itself altering membrane potential. BARKER describes similar actions of peptides (see also Ziegläansberger, *op. cit.*). In Dunn's scheme these substances would have to be called neurohormones, even though they might act synaptically. Thus, I believe that both action and morphology have to be included to encompass commonly held (albeit diverse) concepts of neurohumors.

As BUTCHER well states, the function of a neurochemical cannot be divorced from the spatiotemporal matrix within which it operates.

It is important to emphasize, as have BUTCHER and PALAY, that a given substance may play different neurohumoral roles in different places. I did not imply otherwise, and I agree with suggestions that my proposed categories should describe functions (DREIFUSS & HARRIS; ELLIOTT & BARCHAS; WILSON).

The broad categories that I have suggested are intended only to provide a common ground for communication. Even if numerous categories were exquisitely defined, they would not adequately characterize neurohumoral functions. Characterization requires considering the actions of a neurohumor in the context of the anatomy and physiology of the particular system examined (see BUTCHER; FREEDMAN; WOODWARD ET AL.). BOULTON, BOYLE, and LIBET suggest useful sets of characteristics that might be used to distinguish actions of neurohumors.

Other reasonable schemes for categorization are of course possible. (see SMITH and TRACZYK, for example). BUTCHER cogently argues that substances that do not themselves produce observable actions but modify responses to transmitters might be called neuromodulators. HOYLE makes "direct action" versus "modulation" the primary distinction among neuroactive substances (my neurohumors). Given the complexity of electrophysiological responses, I am not sure how easy it will be to distinguish direct from modulatory or indirect actions. ELLIOTT & BARCHAS make a similar distinction. In contrast, several other commentators, particularly DUNN, IVERSEN, LIBET, RYALL, and TRUMAN concur with my opinion that it is more useful to speak of modulation as an action, but not to use the word as a noun – that is, not to categorize substances as modulators. However, the differences of opinion among the two groups may not be as great as they may seem. From HOYLE's description, for example, I get the impression that he is primarily concerned with classifying *actions* of neuroactive substances.

There is also some divergence in commentators' concepts of what constitutes modulatory action. PHILLIS would call a neurohumor a modulator if it causes long-lasting alterations in neuronal excitability or transmitter release, and a transmitter if it mediates rapid transmission. However, like HOYLE, LIBET, and RYALL, I do not find time course of action a sufficient basis for distinction, because in some systems direct excitation or inhibition may operate relatively slowly. Kupfermann's thoughtful review (cited by IVERSEN) had not appeared when I wrote my article, but I agree with his emphasis on a combination of time course and contingency as the primary characteristics defining modulation. This is congruent with the common language use of the word, as several commentators point out (see HOYLE; LEMBECK; and PALAY). Palay reiterates my position that there is no utility at this time in trying to tie modulation to particular mechanisms. The discussions of complex electrophysiological responses by BARKER, BULLOCK, FREEDMAN, LIBET, WEIGHT, and WOODWARD ET AL. make it clear that diverse mechanisms are involved, and that we are not now in a position to assign them categories.

FLOREY suggests a unique approach to distinguishing modulator substances from transmitters, based more on the way in which they are released than how they act. I wish he had expounded further on this suggestion, so that we could better compare it to the concept of contingency of action discussed by other commentators. Florey's definition seems relevant to the previously discussed possibility of neuroactive substances co-existing in neurons, one released to modulate the action of a primary transmitter.

Incidently, it seems that many of FLOREY's objections might have been obviated by a more careful reading of my paper. For example, he claims that I ignore the fact that several forms of transmitter action have been recognized and several types of inhibition and excitation have been distinguished. In fact, I spent the major part of the long section on complex electrophysiological responses discussing those varied forms. I do not speak of "simple inhibition" or "simple excitation," as he misquotes me, but I review a range of evidence about "complicated responses that are not simply excitation or inhibition, as conventionally defined." He states that I omit the action of the transmitter substance from my definition of synaptic

transmitters, yet the third sentence of that definition explicitly talks about action. He has me saying that Sherrington's use of the word "synapse" was prescient, when what I actually said was that Sherrington "recognized, almost presciently, that crucial features of the operation of neuronal circuits could be explained by the character of synaptic transmission."

FLOREY accuses me of unwarranted teleology in my definitions. However, they are teleological only in the sense that specific functions (e.g. alteration of the activity of target cells) are attributed to certain events (e.g. release of neurohumors). This is a common use of language and one difficult to avoid. For example, I would respond to BUTCHER's objection that my definition of neurohumor does not include actions at autoreceptors by saying that the primary function of neurohumors is to alter activity in other cells, and that action at autoreceptors is secondary and subordinate. This does indeed have a teleological flavor, and philosophers of science have discussed how this flavoring creeps into every discussion of biological function, but I believe that this should be the subject of a different issue of *BBS*.

Nonsynaptic release

COSCINA claims that I fail to adequately criticize the methodology employed by various authors reporting apparent nonsynaptic release sites. This assertion is hard to understand, since half of the section (the longest in my paper) that asks whether transmitters can be released nonsynaptically is devoted to comparing results from different laboratories and exploring possible reasons for the differences observed. In an article written for readers of diverse specialities, it would not have been effective and would have taken an inordinate amount of space to have made a highly technical review of methodological problems. Curiously, Coscina failed to use the opportunity provided by his own commentary to make the methodological critique he finds so important.

In my article I concluded that nonsynaptic release is an important possibility to explore, but its occurrence has yet to be clearly established in the mammalian CNS (other than in the neuroendocrine system). The difficulties of obtaining conclusive evidence in the CNS are attested to by the comments of ARLUISON about dopamine release in the substantia nigra, and OSBORNE about interpreting EM-autoradiographical findings. Osborne raises an interesting question: If nonsynaptic varicosities release transmitters, why would they have high affinity uptake sites for their own transmitters, since these uptake sites are thought to be a mechanism for inactivating transmitters in synaptic clefts, and it would not seem desirable to inactivate a transmitter before it could diffuse away to its remote targets. It would be interesting to know whether the identified nonsynaptic release sites in invertebrates have high-affinity uptake mechanisms.

IVERSEN rightly points out that it would be surprising if release did *not* occur from some CNS terminals lacking close synaptic apposition, since such release sites are commonly found in the peripheral nervous system (and in some invertebrate systems). Let me reemphasize the point made in my original article that nonsynaptic release does not necessarily imply the broadcasting of transmitter to distant targets. I agree with OSBORNE's comment that complex electrophysiological actions are not necessarily an indication of nonsynaptic release; however, the striking association of such actions with nonsynaptic release in invertibrates makes it quite reasonable to search for analogous arrangements in the mammalian CNS.

BROWN and WILSON raise the interesting possibility of another aspect of unconventional transmission: nonvesicular release. I would have liked to have seen more discussion of that possibility.

Dale's Principle

Several commentators caution us about prematurely concluding that Dale's Principle is violated in some neurons. YORK warns that immunohistochemical techniques require great care to demonstrate specificity of the neurochemical localized. The presence of a putative neurotransmitter in neurons does not necessarily imply that it was locally manufactured, since specialized uptake systems abound (HATTON), and their functions are only partly understood. Nor does co-existence necessarily imply functional release of both species of molecule (IVERSEN). There is some evidence of release of more than one neuroactive substance from the same neuron (Cottrell *op. cit.*), but it remains to be established whether both substances act as transmitters (OSBORNE).

DUNN observes that even though our attention has focused on the co-existence of neuroactive substances in the same neurons, an important tenet of Dale's Principle is that each axonal branch releases the same products. Thus, OCHS's suggestion that there exist mechanisms whereby different transmitters (or precursor materials) could be delivered to different terminals is intriguing. It was not clear from his comments whether experimental evidence already exists for such routing of transmitter materials.

It is inherently impossible to prove Dale's Principle universally correct, since we can at best test what products are released from a few branches of a few neurons. The practical question is how many exceptions would be required to unseat our acceptance of the principle as a working assumption about neurons.

Behavioral implications

Nonconventional modes of transmission will certainly have implications for our understanding of the behavior of organisms. In my article I tried to portray something of the flavor of speculations that have been made about such implications. Admittedly, speculations about mammalian functions, including my own, are still too generalized to be testable as hypotheses. Invertebrate preparations have provided one major approach to elucidating the neuronal mechanisms of behavior. KUPFERMANN points out a satisfying confluence of concepts of neuronal modulation with behavioral modulation in the actions of serotoninergic neurons in *Aplysia*, and BRANTON & MEYERI provide an instructive comparison of the operations of egg-laying hormone with that of the locus coeruleus in mammals. EVANS and HOYLE discuss the related example of octopamine released nonsynaptically from an identified locust neuron. At present, these systems provide a more concrete basis than mammalian nervous systems for discussing how nonjunctional transmitters (or neurohormones) may operate to coordinate large neuronal ensembles and switch among behavioral modes.

CHUTE's assertion that my discussion of behavioral states or modes revives old nonconnectionistic or field theories of brain function is startling and incorrect. First of all, I do not subscribe to Lashley's early notion of equipotentiality, and it is now well established that functions of the cerebral cortex combine both distributed and localized aspects, an expression in part of the ubiquitous interplay of convergence and divergence of neuronal circuitry. Secondly, the switching of behavioral modes by nonjunctional neurotransmitters does not imply any sort of field theory. The commentators who described such switching in invertebrates found no need to invoke field theories, and my impression of the neuroanatomy of invertebrate preparations is of "hard-wiring." Chute may have misunderstood my meaning in discussing regulation of the state of behavioral responsiveness, because, contrary to his assertion, such phraseology is very much alive and well in the literature (see Hobsen and Scheibel, *op. cit.*).

ITO asks how temporary modulation of the efficacy of synaptic transmission has implications for learning and memory (see also WEIGHT). I do not want to spend much space on this, since my ideas are pure speculation. However, it has been established that modulatory substances can facilitate either transmission in a single synaptic path or associative pairing of convergent synaptic inputs. We may speculate that each transient facilitation may induce some more enduring intracellular molecular event (e.g. protein synthesis, phosphorylation, or methylation) with the cumulative net effect of a long-lasting change in synaptic efficacy. Such changes could provide a basis for memory, as originally suggested by Catherine Hebb.

Many of us have espoused Hebb's hypothesis in the ensuing thirty years, but, sadly, no one has been able to devise an adequate experimental test.

In response to ITO's other query, I did not mean that neurons are elevated from simple devices to complex computers by nonjunctional transmission alone, but by the whole gamut of complex processes alluded to in my article. Ito adds an intriguing dimension to this discussion with his suggestion of possible "house-keeping" functions of systems such as the diffuse projections of the locus coeruleus. I would like very much to see this suggestion elaborated.

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