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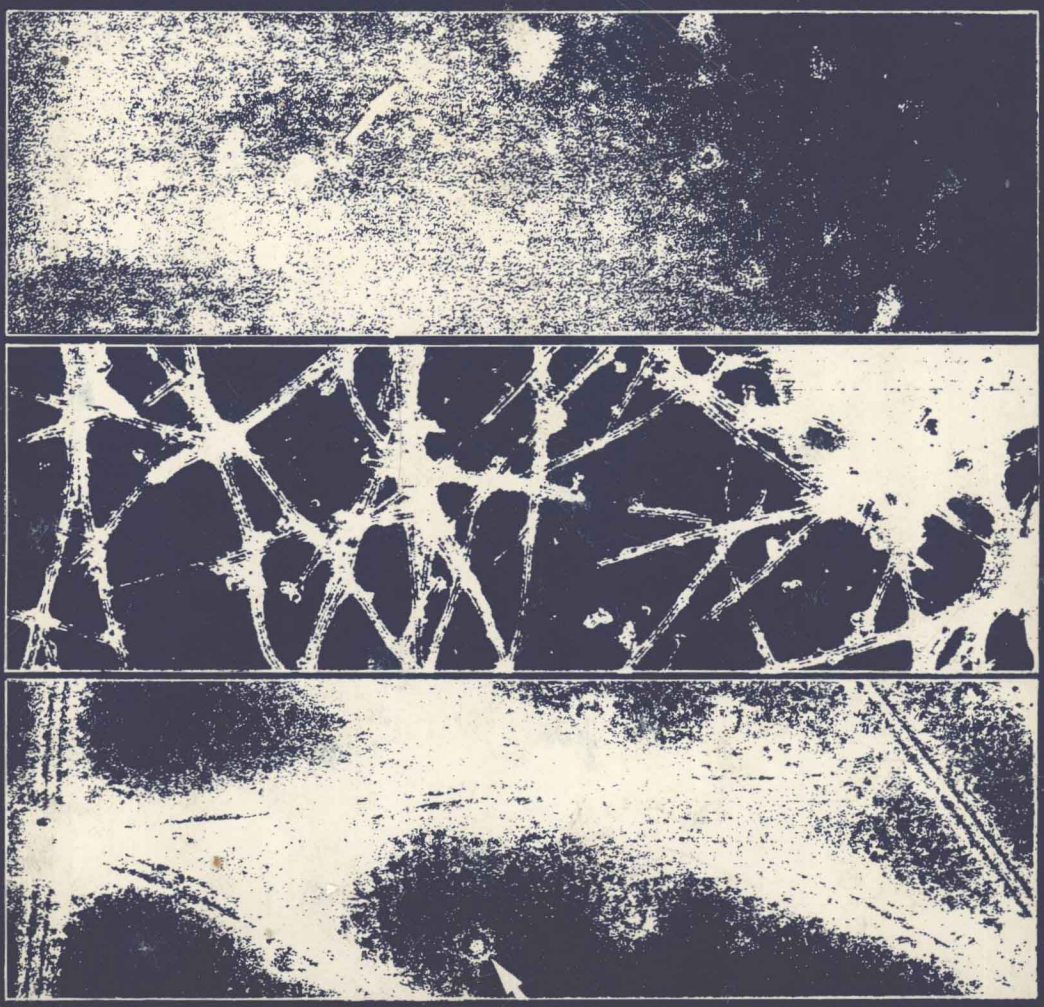
Regulatory Mechanisms of Synaptic Transmission



Edited by Ricardo Tapia and Carl W. Cotman

Regulatory Mechanisms of Synaptic Transmission

Edited by

Ricardo Tapia

Department of Neurosciences
Center for Research in Cellular Physiology
Universidad Nacional Autónoma de México
Mexico City, Mexico

and

Carl W. Cotman

Department of Psychobiology
University of California
Irvine, California

Published in cooperation with
Universidad Nacional Autónoma de México

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Regulatory Mechanisms of Synaptic Transmission

PREFACE

This book, which originated in the presentations of a symposium sponsored by the Universidad Nacional Autónoma de México, held in Mexico City on April 14-16, 1980, represents an attempt to analyze some of the most relevant aspects of synaptic transmission. This topic was chosen on the strong belief that the progress of the neurosciences depends to a great extent on the understanding of the basic mechanisms of synaptic function. Rather than selecting only a specific approach or speciality, the book intends to cover this field in a multi-disciplinary way. This means that neurochemical, neurophysiological and morphological studies are mingled throughout the book, which hopefully will help the reader to integrate the different faces of the same problem.

Across the book the presynaptic component of synaptic transmission and its regulation is stressed much more than the postsynaptic phenomena. Although this might be a limitation, it has the advantage of increasing the focus of the book on a series of events which are gaining importance and interest every day.

The book covers several aspects of the synaptic role of three amino acids, glutamate, γ-aminobutyrate and taurine, as well as that of catecholamines and some peptides, emphasizing their regional distribution and possible participation as neurotransmitters or modulators. The mechanism of the action of calcium in neurotransmitter release is amply revised both neurophysiologically and neurochemically, including the participation of some intraterminal proteins. Synaptic plasticity is analyzed through the study of selective reinnervation of muscle fibers and of simple forms of learning. Certain more integrative aspects, such as presynaptic inhibition, facilitation by repetitive stimulation and synaptic mechanisms in sleep, are also covered. Several of the chapters include theoretical models based on the available experimental data.

We wish to express our gratitude to the Centro de Investigaciones en Fisiología Celular, the Secretaría Ejecutiva del Consejo de Estudios de Posgrado and the Dirección General de Asuntos del Personal Académico, all of the Universidad Nacional Autónoma de

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The Editors

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George R.Siggins

Arthur V. Davis Center for Behavioral Neurobiology

The Salk Institute, La Jolla, California 92037

INTRODUCTION

In a meeting devoted to the discussion of synaptic regulation, it seems instructive first to address the issue of what kinds of synaptic messages may be transmitted and what criteria may be developed to define the different types. This task seems particularly timely now because of the burgeoning number of substances found in brain which seem not to strictly fit our preconceived notions of a neurotransmitter. After counting all the peptides, monoamines, amino acids, nucleotides, prostaglandins and steroids that have been advanced as transmitter candidates, I come up with a number exceeding 60. It seems unlikely that such a wide variety of substances would provide only a few chemical messages. Therefore, after developing criteria for neurotransmitters, neuromediators, and modulators, I will provide examples of substances that could satisfy the criteria for these classifications, with special emphasis on norepinephrine (NE), cyclic AMP and the opioid peptides.

Historical Perspective

The hypothesis of chemical neurotransmission was first developed for the vertebrate peripheral nervous system, which was thought to include nerve terminals that communicated to muscle tissue via release of acetylcholine or catecholamines (17,18,24,54). The chemical doctrine of Dale was later generalized to the central nervous system, although not without vigorous discussion along the way (cf. 10,19). Arising out of the controversy and research in this area was the principle that different chemicals and different neurons transmit different messages (excitation or

inhibition). Most of the resistance to the doctrine of central chemical transmission seems to have arisen out of consideration for the speed by which spinal cord synapses transmitted their messages. Sir John Eccles, then a critic of the doctrine, felt that central messages demanded rapid electrical transmission, although later studies have shown that the original peripheral models for the chemical doctrine (the autonomic nervous system) showed both rapid and slow forms of chemical communication (12,92). The Eccles group performed the experiments that eventually proved the chemical doctrine for the spinal cord (16,26). However, the narrow view that persisted until recently was that neuro-transmission operated in only two modes, fast excitation via excitatory postsynaptic potentials (EPSPs) and fast inhibition via inhibitory postsynaptic potentials (ISPSs), both caused by the opening of conductance channels in the post-synaptic membrane (see below and refs. 25,44), brought about by the release of fast-acting transmitters from large, rapidly conducting axons.

However, results from more recent electrophysiological and ultrastructural studies on autonomically innervated smooth muscle systems point up the fact that neurotransmission need not always involve fast signals transferred across narrow, specialized synaptic clefts. Features common to many neuro-effector smooth muscle systems (see 12,13,52,76) are: 1) long junctional delays; 2) long time courses of post-junctional potential changes; 3) the requirement for repetitive nerve activation to produce detectable, summated responses; 4) wide junctional gaps of up to thousands of angstroms, without synaptic specializations; 5) transmitter release from boutons or varicosities 'en passage.'

It is now apparent that nerve cells also communicate with one another by means of a much more complex vocabulary than a simple rapid 'yes' or 'no'. Inklings of the idea that neuronal transmission might not always require the rapid opening of conductance channels was seen in the non-synaptic models of the photoreceptor (4) and the stretch receptor (60), where 'leaky' ion channels seemed to be closed or 'inactivated' to produce the physiological response. The possibility that such a novel form of response might apply also to vertebrate central neuro-transmission gained credence with results of studies on mammalian and amphibian sympathetic ganglia. Here, a new form of slow transmission, the slow IPSP (sIPSP) and the slow EPSP (sEPSP), occurred without an increase in membrane conductance, and co-existed with the more usual form of rapid nicotinic depolarization (EPSP) accompanied by a conductance increase (27,65). Although a controversy still centers on whether the sIPSP is generated by a decrease in ionic conductance (96) or by activation of an electrogenic pump (65), the important point is that investigators began to ask if such a new form of transmission might not also apply to the central nervous system.

Shortly after the discovery of the sIPSP and the sEPSP, it became apparent that similar slow mechanisms might apply also to certain forms of synaptic transmission in brain. Thus, exogenous norepinephrine (NE) and activation of NE-containing fibers was found to evoke slow hyperpolarizations in cerebellar Purkinje cells associated with either a decrease or no change in ionic conductance (40; also see below), while muscarinic, cholinergic depolarizing responses associated with a decreased conductance were reported in spinal cord (105) and in a cortical area thought to possess cholinergic fibers (50). These responses in central neurons thus parallel those in sympathetic ganglia, where the sEPSP is thought to arise from activation of muscarinic receptors (27,47), while the sIPSP may be generated by release of a catecholamine (27, but see ref 97).

However, at about this time researchers began to anticipate the presence in brain of even more complicated forms of neuronal communication. For example, hypotheses were advanced that the post-synaptic action of certain neurotransmitters (NE, ACh) might be mediated by the generation of cyclic nucleotides (cyclic AMP and cyclic GMP; see below). In addition, with the discovery of a wide array of neuroactive peptides in brain came the likelihood that several of these might alter the responses of neurons to other neurotransmitters without having a direct action of their own. Examples of this putative form of neuronal communication were seen in iontophoresis experiments showing that TRH could alter the excitatory action of ACh (99, but see 75), and that opioid peptides could reduce synaptic efficacy and glutamate and ACh responses (2,100,102); in both cases interactions occurred without an apparent direct peptide effect on neuronal excitability.

Measures of Excitability: Transmembrane Properties

As suggested above the most conventional mechanism of hormone or transmitter action on cell excitability involves binding to a specific receptor, resulting in a change in the transmembrane permeabilities to one or more ions. The influence on membrane potential depends upon the ionic permeability changes. Each ionic species is in unequal concentration on either side of the membrane due to the relative membrane impermeability to some ions and the activity of ion pumps; thus there is a driving force, determined by the concentration gradient, for each ionic species. An equilibrium potential, E_X, can be defined for each ion (X) at which the electrical gradient is exactly equal to the chemical gradient for that ion by the Nernst equation:

$$E_X = \frac{RT}{F} \cdot \frac{\ln (X_i)}{(X_o)}$$

where R is the universal gas constant, T is absolute temperature,

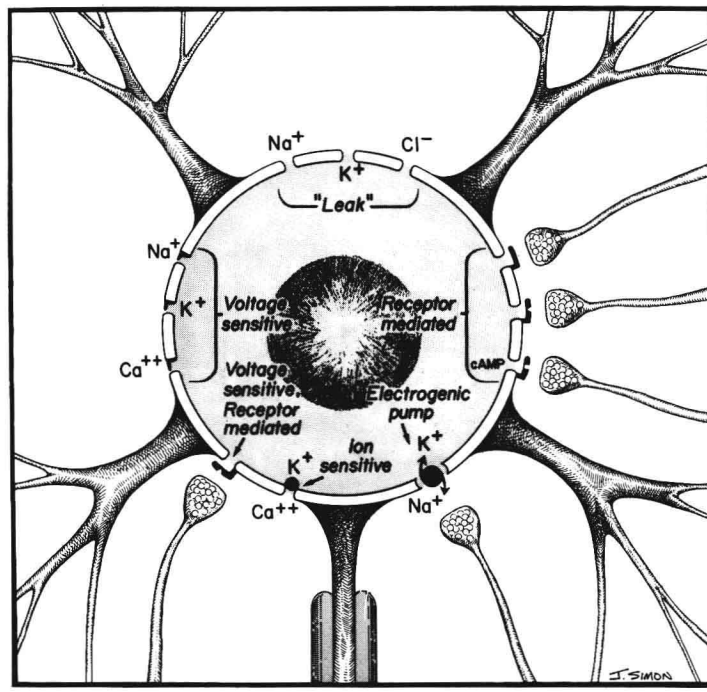

Fig. 1. Schematic representation of several types of ion channels and related processes thought to contribute to the electrical activity of neurons. Most neuronal electrical activity is generated by the flow of ions through such conductance channels. Those which are always open (top of figure) are termed 'leak' conductances; these, especially those for potassium and sodium ions, contribute largely to the resting membrane potential. The receptor-mediated channels are depicted on the right. These are activated or inactivated by chemicals (most neurotransmitters) and are thought to account for the conventional non-voltage-dependent or passive responses to activation of synaptic pathways. The traditional voltage-dependent channels (left) are those which are open only at certain membrane potentials; these are usually thought to contribute to the generation of an action potential, after the membrane potential is brought to a threshold (trigger) level of depolarization by injected current or activation of synaptic inputs. One result of a voltage-sensitive conductance is shown at the bottom left, wherein the entry of calcium during the action potential triggers the efflux of potassium ions, resulting in membrane repolarization (and sometimes the hyperpolarizing afterpotential) at the conclusion of the action potential. Such ion sensitive channels are opened only when a particular ion (e.g. calcium) is present. A relatively new development is the existence of voltage sensitive, receptor-mediated ion channels (lower left); as with conventional synapses, such channels may be

F is the Faraday constant, and the subscripts i and o indicate inside and outside concentrations of the ion (see 25,44).

Fig. 1 is a schematic representation of the types of ionic channels likely to generate the equilibrium potentials for the major ionic species involved in determining resting transmembrane potential (RMP), action potentials and receptor mediated potential shifts in excitable cells. The approximate range of ionic equilibrium potentials involved in determining membrane potentials for most excitable cells are: Ca^{++}, +20 to +50 mV; Na^+, +20 to +40 mV; Cl^-, -10 to -80 mV; K^+, -40 to -80 mV. The resting membrane potential usually can be approximated by the sum of the contributions of Na^+, K^+ and Cl^-; thus most excitable cells display RMPs in the range of 40-100 mV, internally negative. Under resting conditions, K^+ permeability usually predominates and RMP is relatively near but slightly positive to E_{K^+}. When a hormone, neurotransmitter or second messenger increases permeability to a single ion, the potential will approach the equilibrium potential for that ion. If such an agent causes a decrease in the permeability to one ionic species, the potential will move away from the equilibrium potential for that ion toward that of the ion with the dominant permeability. Thus, an increase in K^+ permeability will generally hyperpolarize, whereas an increase in Na^+ permeability will depolarize, an excitable cell. In most brain neurons, E_{Cl^-} is more negative than RMP; here permeability increases to Cl^- are hyperpolarizing. In the vertebrate neuromuscular junction and many synapses mediating fast excitation the transmitter opens a channel for both Na^+ and K^+, pushing the potential at the neuromuscular junction to about -15 mV, approximately midway between E_{K^+} and E_{Na^+}.

However, it is not clear what changes in ionic conductance, if any, would appear if ionic pumps were the cause of excitability changes (cf. 95). Such electrogenic pumps are thought by some to

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opened or closed by neurotransmitters, but only at certain membrane potentials. Since many or most voltage-dependent conductances are associated with action potential mechanisms, activation of such receptors would be expected to alter properties of the spike (see text). Activation or inhibition of electrogenic ion pumps (lower right) could also contribute to receptor- or non-receptor-coupled changes in membrane potential. Generation of cyclic nucleotides, by nucleotide cyclases, possibly through activation of transmitter receptors (see text for example of cyclic AMP), could open or close ion channels directly, or alter voltage sensitive conductances or membrane pumps, thus significantly altering neuronal excitability (Taken from ref. 84).

account for certain slow synaptic potentials in sympathetic ganglia (see 64). Moreover, electrophysiological studies of vertebrate and invertebrate neurons suggest that some neurotransmitters can alter excitability without directly changing membrane potential. For example, in some neurons where E_{Cl^-} is very near RMP, transmitter-increased permeability to Cl^- would produce no potential change, but any other synaptic inputs would be reduced in effectiveness because of the 'shunting' effect of the reduced resistance. Conversely, a neurotransmitter which reduces ionic permeabilities (increasing resistance and therefore the 'voltage drop' for a given second synaptic current) would increase the responsiveness of neurons to other synaptic inputs.

Another recently described phenomenon of importance is the voltage-dependent action of some hormones and neurotransmitters. Here, concentrations of the agonists often below those normally producing potential changes are capable of altering ionic permeabilities which are usually not active at the RMP. For example, alterations of spike threshold have been seen with GABA and opioid peptides in some cultured spinal neurons (1). Since spikes or action potentials involve membrane conductances which are activated by changes in membrane voltage and these actions are blocked by specific receptor antagonists, such a novel phenomenon could be ascribed to a receptor-mediated voltage-sensitive conductance change (Fig. 1). Receptor-mediated changes in voltage-dependent calcium channels are of even greater interest because calcium has so many regulatory functions upon a variety of cellular activities (e.g., secretion, calcitonin activation, genome expression, cell motility). Examples of this phenomenon are the decreases in the late (calcium) plateau component of the spike in mammalian dorsal root cultures produced by GABA, norepinephrine, serotonin and enkephalin (23) and in rat sympathetic nerves by norepinephrine (42). Activation or prolongation of voltage-sensitive Ca^{++} channels by serotonin has been observed in aplysia neurons (45,71; see Klein, this volume).

Furthermore, Brown and Adams (11) report a muscarinic cholinergic action (sometimes depolarizing) on neurons of sympathetic ganglia which arises from inactivation of a voltage-dependent K^+ channel. This latter action is similar to one action of catecholamines on cardiac Purkinje fibers, where the slow inactivation of a voltage-dependent K^+ conductance is responsible in part for a depolarizing pacemaker potential. By speeding the inactivation of this channel, norepinephrine increases the frecuency of firing. In addition, norepinephrine (and cyclic AMP as well) appears to enhance the slow inward current (mostly calcium) of the cardiac potential and the outward K^+ current responsible for repolarization; both of these effects involve voltage-sensitive conductances.

It is against this basic and historical backdrop that an

attempt will be made to develop criteria to be used for identifying neurotransmitters, neuromodulators and mediators in nervous tissue. Where possible, I will suggest broad criteria with sufficient latitude for inclusion of possible undiscovered new forms of neuronal communication, rather than be forced to generate new terms for a process that might still be best described as, for example, neurotransmission. In this effort, the features of peripheral neurotransmission will be kept in mind as continuing models for central communication.

NEUROTRANSMITTER CRITERIA

My personalized criteria for identification of a neurotransmitter may be paraphrased and condensed from the several criteria previously suggested (98):

1. Neuronal localization of the substance and its enzymes of synthesis and degradation.

2. Release of the substance upon selective activation of a specific neuronal pathway.

3. Identical physiological response to exogenously applied transmitter and to activation of the pathway.

4. Identical action of pharmacological agents (antagonists, etc.) when tested against the effects of exogenous transmitter or of the activated pathway.

To some, these criteria may show a lack of concern for the ultrastructural bases of neurotransmission (e.g., the presence or lack of synaptic 'specializations'). However, such considerations were omitted because they are still very controversial, because they have been discussed in detail elsewhere (7,8,21), and because of continued difficulties in documenting correlations between structure and physiology. Moreover, the possibilities for a broad array of morphological forms of neurotransmission in CNS may be indicated in the peripheral nervous system: some sympathetic boutons transmit direct messages without synaptic specializations to their smooth muscle contacts in an 'en passage' fashion, but do show them at intraganglionic nerve-nerve contacts (41).

It will be noted that there is no mention in the stated criteria of the speed of transmission. Thus, as in peripheral systems, a slowly acting substance is just as much a neurotransmitter as a fast acting one. Moreover, a substance is not excluded as a neurotransmitter because it also alters (or 'modulates') the response of another neurotransmitter. If this were the case most currently-conceived neurotransmitters would be excluded because they alter

membrane conductance (or resistance), and the result of this would likely be altered potentials generated by other synapses or transmitters. For example, GABA and classical IPSPs inhibit cells by increasing conductance to Cl^- and perhaps K^+ ions; such an increase in conductance would cause a decrease in the membrane such that a given synaptic current generated by another input to the cell would generate a smaller potential shift across the reduced resistance. Thus, GABA might be expected to inhibit firing by two mechanisms, hyperpolarization and reduction of other synaptic potentials. By the same reasoning, application of glutamate or activation of a fast EPSP (increasing conductance to Na^+ and reducing membrane resistance) might be expected to excite cells by depolarization, but reduction of other synaptic potentials should also be considered.

The word 'response' in criterion three above was purposely chosen as a broad term, in order to cover a wide spectrum of possible direct mechanisms of transmitter action on cell excitability. Thus, substances which hyperpolarize or depolarize by increased or decreased conductance, or by activating or inactivating electrogenic pumps, or which alter spike thresholds (with or without potential changes), perhaps via effects on voltage-dependent conductances, will all be considered neurotransmitters by these criteria because they directly affect neuronal excitability.

MEDIATOR CRITERIA

A mediator of neuronal communication might be best exemplified by the role of cyclic nucleotides as 'second messengers.' The second messenger concept as currently applied to brain has evolved from the mediator role of cyclic AMP in peripheral hormonal responses, as first suggested by Sutherland et al. (1965). Modified for neuronal transmission or local modulation (see below), this concept may be summarized as follows:

A synaptically released neurotransmitter or locally released neuroactive agent could act at certain pre- or postsynaptic receptors to activate adenylate cyclase and the synthesis of cyclic AMP. Intracellular cAMP would then initiate subsequent enzymatic or molecular events, which, among other actions (e.g. long-term trophic effects) could result in changes in membrane potential and cell discharge rate. Four major criteria may be adapted from the criteria for hormones, to establish that the action of a transmitter is mediated by a cyclic nucleotide (5,6,83).

1. Exogenous neurotransmitter substance and activation of the synaptic pathway both regulate intracellular levels of cyclic nucleotide in the postsynaptic cell.

2. The change in intracellular cyclic nucleotide content should precede "the biological event" triggered by the transmitter or nerve pathway.

3. Responses to the transmitter or nerve pathway should be logically altered by drugs that specifically interact with the nucleotide cyclase or that inhibit the appropriate phosphodiesterase.

4. Exogenous cyclic nucleotides (and analogues which activate protein kinase) should elicit a response identical to the biological event caused by the transmitter or nerve pathway.

Unfortunately, attempts to satisfy the second messenger criteria for central neurons meet with considerable technical obstacles, such as the indirect actions of systemic drugs, blood-brain barriers to systemic agents, slow nucleotide sampling and measurement times compared to fast synaptic events, and relative impermeability of cyclic nucleotides into target cells. Several of these obstacles can be partially overcome in the central nervous system by the techniques of microiontophoresis and electrophysiology, as they have been applied to several brain areas (see below).

In spite of these difficulties, an important new consideration with respect to the likelihood that cyclic nucleotides mediate the function of neurotransmitters is the idea that a neurotransmitter can generate potential and conductance changes not by altering passive membrane properties but by altering neuronal metabolism (or energy function) which in turn alters transmembrane properties. This constitutes the "energy" dimension of neurotransmission as seen by Bloom (7).

MODULATOR CRITERIA

The term modulator has received increasing attention recently as a catch-all category capable of including the action of all substances whose actions differ from those of the classically-conceived spinal cord transmitters. Indeed, references to 'modulators' in the literature seem to be increasing at a frequency in direct correlation to the discovery of new neuroactive substances; the variety of definitions of the term 'modulator' seems almost as numerous as the number of new brain substances. These definitions range from an emphasis on a long time-course of action (31), to any substance (e.g., CO_2 or NH_3) released from non-synaptic sources (28). However, a criterion common to most definitions is the notion that a modulator should have no direct effect of its own, but that it can alter the response of the post-synaptic neuron to other synaptic afferents (2,3). Here again, I will apply a broad definition of modulator, using primarily