

NEUROBIOPHYSICS

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INTRODUCTION

The distinctions between neurobiophysics and other areas of neuroscience are becoming progressively more difficult to discern. Attempts to understand how the nervous system functions have been made from a variety of different scientific perspectives, and a consensus approach appears to be developing that involves some mathematical modeling and attempts to elucidate physical principles, but which is more focused on elucidating physical mechanisms.

1. STRUCTURE AND FUNCTION

Recent evidence suggests that gap junctions play an important role in regulation of the nervous system. Gap junctions are composed of transmembrane protein subunits called *connexins*, 4 to 6 of which form a pore that constitutes one half of a gap junction, the other half of which is formed by a similar structure on an adjacent cell. Heterology among the connexin subunits allows for differences in permeability, conductance, and gating of gap junctions by voltage or various ligands (Kumar and Gilula, 1996).

Flux through gap junctions in retinal cells was visualized in the form of Ca^{2+} waves induced by electrical, chemical, or mechanical stimuli that caused no change in cell-membrane potential (Newman and Zahs, 1997). The Ca^{2+} waves, which could propagate for

more than 10 s and as far as 180 μm from the initiation site, may constitute a previously unrecognized form of neural communication.

A form of Charcot-Marie-Tooth disease (a hereditary peripheral neuropathy) has been attributed to the production of nonfunctional connexin subunits (Spray and Dermietzel, 1995), thereby underscoring the importance of electrotonic conduction between cells in the functioning of the nervous system.

2. ION CHANNELS

X-ray diffraction of most types of ion-channel proteins is presently not possible because of the difficulty in producing suitable crystals. *Site-directed mutagenesis* studies, however, have provided insight into structural features of ion channels such as the location of the ion-selective filter for voltage-gated Na^+ , Ca^{2+} , and K^+ channels (Guy and Durell, 1995). The filter appears to lie in the channel's pore-lining segments (P segments). By changing specific amino acids in the P segments, the permeant-ion profile of Na^+ and Ca^{2+} channels can be interchanged. Even though the primary structures of K^+ channels are more heterogeneous, location of the selectivity filter has been narrowed down to a three-amino-acid motif in the P segment where mutations have invariably disrupted function (Navarro *et al.*, 1996).

Just as ion channels seem to share a common structural and functional domain for ion

selectivity, all known voltage-gated channels sense voltage changes with a protein subunit called the S4 segment. This segment has been identified as the voltage sensor of K^+ channels on the basis of measurements of the gating current (Mannuzzu *et al.*, 1996). A span of at least seven S4 amino acids was exposed extracellularly during activation of K^+ channels in a *Xenopus* oocyte model system. This suggests that during activation either the S4 amino acids moved extracellularly, or they remained static while the rest of the channel protein moved intracellularly.

The basis of the gating current in Na^+ channels was studied using hydrophilic cysteine-modifying reagents (Yang *et al.*, 1996). The procedure (systematic replacement of each S4 residue with cysteine followed by use of the membrane-impermeant reagents to modify the cysteines and detect any changes during activation) permitted a determination of precisely how many S4 amino acids and their accompanying charges moved from the intracellular to the extracellular compartment during channel activation, and the distance through which they moved. The total gating current was found to consist of the movement of about 10 positive charges through a distance of 4.5–11 Å. Since the cell membrane is normally 40 Å thick, the observation of (at most) only an 11-Å displacement between the extracellular and intracellular compartments suggests that the geometry of the cell membrane is radically altered in the vicinity of ion channels.

3. NEURONAL SYSTEMS

3.1 Dendrites

The role of dendrites was traditionally thought to be limited to that of supporting the passive unidirectional propagation of current, but new evidence suggests that dendrites can also integrate and process bidirectional information. Back-propagation of axon-derived action potentials (*antidromic action potentials*) was demonstrated in the hippocampus (Magee and Johnston, 1997) and neocortex (Markram *et al.*, 1997), and shown to be necessary for long-term potentiation. Subthreshold synaptic inputs that were followed within about 100 ps by the arrival of back-propagating action po-

tentials facilitated amplification of subsequent subthreshold synaptic inputs. Timing differences above the window fundamentally altered the plasticity of the synapses (Sejnowski, 1997). Thus, it appears that dendrites may have an important and previously unrecognized role in synaptic plasticity.

3.2 Neural Networks

Neural networks have been developed as useful interpretative tools. For example, they have been used to determine secondary structure of proteins (Rost *et al.*, 1995), putative coding sites in DNA, and chromosome classification (Leon *et al.*, 1996). Networks can even outperform human beings in some diagnostic judgment tasks, including diagnosis of acute myocardial infarction under the high stress and time constraints of the emergency room (Baxt and Skora, 1996) and interpretation of breast biopsies (Baker *et al.*, 1996). However, the original promise of neural networks, that they could accurately model the actual mechanism of biological learning, has not been fulfilled. It is becoming increasingly more clear that information processing within the central nervous system is more complicated than that embodied in the neural-network approach.

3.3 Volume Transmission

Movement of information in the central nervous system can occur via nonsynaptic mechanisms (volume transmission). One type of such information flow occurs when potentials produced by groups of cells propagate in the electrically conductive extracellular fluid and induce effects in distant cells—for example, synchronizing firing or altering firing probability (Bullock, 1997). The physiological significance of signalling by means of volume electrical conduction is still problematical, but diffusion of neuroactive substances through brain tissue from their point of secretion to distant binding sites is increasingly being recognized as an important mechanism for regulating cell activity.

Neuropeptides (a subset of neurotransmitters) are usually cosecreted with a classic neurotransmitter (biogenic amines, amino acids, and acetylcholine), but at lower levels. Neuropeptides are good candidates for

volume transmitters because they are present in the extracellular fluid for relatively long periods and have relatively high affinity for their receptors. For example, when mossy fibers of the cerebellum were stimulated, dynorphin (a neuropeptide that was cosecreted with glutamate) inhibited the effects of glutamate in unstimulated mossy fiber tracts as a result of diffusion through the extracellular fluid (Weisskopf *et al.*, 1993). Comparable results were found in other studies (Chen *et al.*, 1995; Kolaj *et al.*, 1995).

Nitric oxide (NO) is also a possible signaling agent because it is produced in both neurons and glia and can quickly diffuse through the extracellular fluid. NO depressed voltage- and Ca^{2+} -dependent currents in hippocampal slices from Sprague-Dawley rats (Erdemli and Krnjevic, 1995), again suggesting a mechanism whereby distant groups of cells could interact with one another.

In addition to isotropic diffusion, evidence has been found for the occurrence of preferential extracellular diffusion along fiber bundles within the brain. Channel-like pathways that paralleled myelinated fiber tracts in rats were demonstrated using fluorescent-labeled dextrans and confocal microscopy (Bjelke *et al.*, 1995). The label reached the contralateral side of the neostriatum, indicating that long-range routes were possible.

Classically, it has been taught that neurotransmitters are released through calcium-dependent fusion of neurotransmitter-laden vesicles at neuronal presynaptic membranes (highly specific regions that form one of the boundaries of the synaptic cleft). Atwell *et al.* (1993) showed that *uptake carriers* can reverse direction and actually secrete neurotransmitter (Fig. 1). This secretion is nonvesicular, usually occurs away from the synaptic cleft, does not require Ca^{2+} , and can be demonstrated in glial cells as well as neurons. Thus, the phenomenon of reverse uptake provides a possible source of diffusible neuroactive agents.

3.4 Memory

Memory must somehow be mediated by a relatively permanent physical change in the nervous system, but the responsible mechanisms have not been delineated. One possible explanation involves the irreversible activation

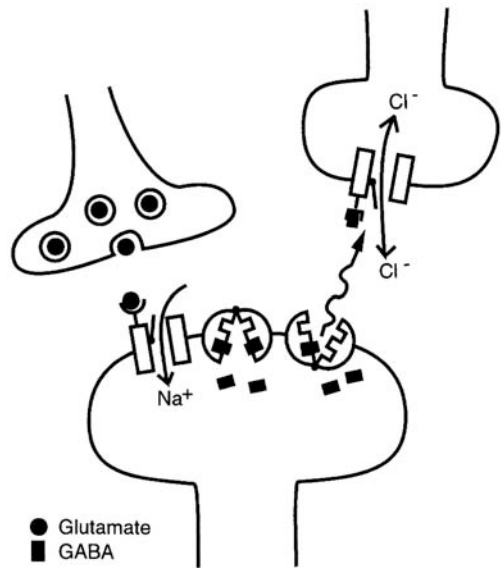


FIG. 1. Reverse uptake and volume diffusion of GABA (Atwell *et al.*, 1993). Glutamate is released by a presynaptic neuron and stimulates its receptors on either glial or neuronal cells, resulting in an Na^+ influx. The increased intracellular Na^+ causes GABA uptake carriers (which normally couple the influx of GABA and Na^+) on the postsynaptic cell to operate in reverse and extrude GABA from intracellular stores. The extracellular GABA can then diffuse and affect other cells having appropriate receptors.

of a calcium-calmodulin-dependent kinase (CaMKII), which is abundant beneath pre-synaptic and postsynaptic membranes. After Ca^{2+} activation, CaMKII can phosphorylate and regenerate itself independent of Ca^{2+} (Fig. 2). Mathematical models have predicted that spontaneous reversion to the inactive state would be highly unlikely, and the active Ca^{2+} -independent state could potentially last a lifetime (Lisman and Goldrign, 1988). The kinase could, therefore, mediate long-term changes in synaptic function by phosphorylating another protein, for example synapsin I (Nayak *et al.*, 1996).

The role of the kinase in mediating memory was demonstrated in animal studies. Mice were genetically engineered to express a permanently activated form of CaMKn (CaMKue) whose expression was regulated by a promoter in the forebrain and by a transactivator (tetracycline) (Mayford *et al.*, 1996). Thus, expression of the transgene (the foreign-DNA gene) was con-

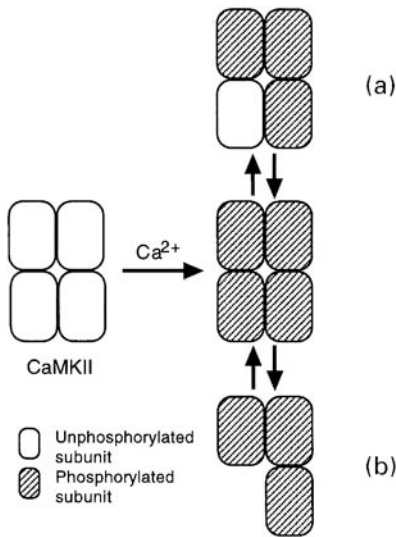


FIG. 2. Irreversible activation of CaMKII (Hanson and Schulman, 1992). In the inactivated state, CaMKII's subunits are unphosphorylated. Stimuli that induce memory activate CaMKII by Ca^{2+} -dependent phosphorylation of the subunits, after which autophosphorylation occurs independent of Ca^{2+} . Thus, if (a) a phosphatase dephosphorylates a subunit, Ca^{2+} -independent activity rephosphorylates it and returns CaMKII to full activity. Furthermore, when (b) a subunit becomes unbound or degraded by normal protein removal processes, the subunit is replaced and phosphorylated independent of Ca^{2+} , resulting in fully activated CaMKII.

finer to the forebrain (the only location of promoter) and was turned off only when tetracycline (T) was added. Tetracycline regulation of the transgene allowed some important observations. The transgenic mice learned and retained memory the same as normal mice when the transgene was inhibited (+T). Expression of the transgene (-T) impaired memory. The impaired memory was reversible by inhibiting the transgene (+T), and memories acquired while the transgene was inhibited could be extinguished by expressing CaMKIIa (-T).

Other studies in which CaMKn was inactivated also showed impairment of various aspects of memory (Glazewski *et al.*, 1996). Thus, inhibiting or activating CaMKn results in significant changes in memory storage, indicating that the kinase is at least partially responsible for information storage in the central nervous system.

GLOSSARY

Antidromic Action Potential: Movement of an action potential in a direction opposite to that of normal propagation. Backpropagation.

Connexin: Protein subunit of a gap junction.

Long-Term Potentiation: A relatively permanent change in a neuronal system.

Site-Directed Mutagenesis: A form of genetic engineering that permits changes in specific nucleic acids in a given gene.

Transgene: A foreign-DNA gene incorporated into an organism's genome early in development, and inherited by offspring in an Mendelian fashion.

Uptake Carrier: Membrane protein that facilitates uptake or release of ions and other substances.

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