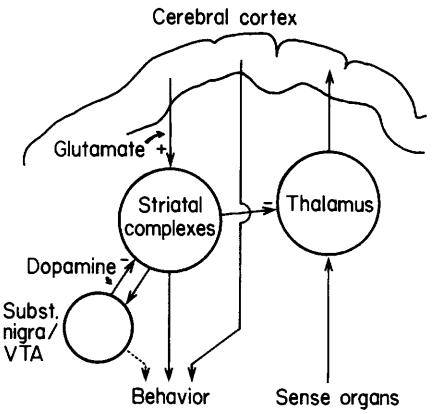

Fig. 6. Schematic representation of the hypothesis that the cerebral cortex can protect itself from an overload of information and from hyperarousal by means of feedback loops engaging the striatal complexes and the thalamus (as well as the mesencephalic reticular formation, not shown). The feedback loops are postulated to be modulated by the mesencephalostriatal dopaminergic pathways. [From (*31*)]

This agent, M100,907 has been characterized as a 5HT2A receptor antagonist. We have found it to work in low dosage in an animal model of psychosis that can be induced by lowering glutamatergic function (*37–39*). Recently this compound has also been found to possess antipsychotic properties in schizophrenic patients (*40*). These and other observations support the view that in addition to dopamine, glutamate and serotonin are also critically involved in schizophrenia. It is likely that other neurotransmitters, such as noradrenaline, acetylcholine, and γ-aminobutyric acid (GABA), will soon be added to this list.

References

1. A. Carlsson, P. A. Shore, B. B. Brodie, *J. Pharmacol. Exp. Ther.* **120**, 334 (1957).
2. A. Carlsson, N.-Å. Hillarp, *Kungl. Fysiogr. Sällsk. Lund förhandl.* **26**, 1 (1956).
3. Å. Bertler, A. Carlsson, E. Rosengren, *Naturvissenschaften* **43**, 521 (1956).
4. A. Carlsson, E. Rosengren, Å. Bertler, J. Nilsson, in *Psychotropic Drugs*, S. Garattini, V. Ghetti, Eds. (Elsevier, Amsterdam, 1957), p. 363.
5. A. Carlsson, M. Lindqvist, T. Magnusson, *Nature* **180**, 1200 (1957).
6. _____, B. Waldeck, *Science* **127**, 471 (1958).
7. A. Carlsson, *Pharm. Rev.* **11**, 490 (1959).
8. _____, M. Lindqvist, T. Magnusson, in *Ciba Symposium on Adrenergic Mechanisms*, J. R. Vane, G. E. W. Wolstenholme, M. O'Connor, Eds. (Churchill, London, 1960), pp. 432–439.
9. A. Carlsson, in *The History of Neuroscience in Autobiography*, L. R. Squire, Ed. (Academic Press, San Diego, CA, 1998), vol. 2, pp. 28–66.
10. _____, B. Falck, N. Å. Hillarp, *Acta Physiol. Scand.* **56** (suppl.), 196 (1962).
11. A. Carlsson, N. Å. Hillarp, B. Waldeck, *Acta Physiol. Scand.* **59** (suppl.), 215 (1963).
12. A. Dahlström, A. Carlsson, in *Discoveries in Pharmacology*, M. J. Parnham, J. Bruinvels, Eds. (Elsevier, Amsterdam, 1986), vol. 3, pp. 97–125.
13. A. Carlsson, *Annu. Rev. Neurosci.* **10**, 19 (1987).
14. _____, M. Lindqvist, *Acta Pharmacol. Toxicol.* **20**, 140 (1963).
15. A. Carlsson, in *Schizophrenia*, S. R. Hirsch, D. R. Weinberger, Eds. (Blackwell, Oxford, 1995), p. 379.
16. A. Carlsson, K. Fuxe, U. Ungerstedt, *J. Pharm. Pharmacol.* **20**, 150 (1968).
17. A. Carlsson, H. Corrodi, K. Fuxe, T. Hökfelt, *Eur. J. Pharmacol.* **5**, 357 (1969).
18. P. B. Berntsson, A. Carlsson, H. R. Corrodi, *Composés Utiles Tant qu'Agents Anti-dépressifs, et Procédé pour Leur Préparation*, Belgian Patent No. 781.105 (1972).
19. A. Carlsson, M. Lindqvist, *J. Pharm. Pharmacol.* **21**, 460 (1969).
20. A. Carlsson *et al.*, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **275**, 153 (1972).
21. W. Kehr *et al. J. Pharm. Pharmacol.* **24**, 744 (1972).
22. A. Carlsson, in *Chemical Tools in Catecholamine Research*, O. Almgren, A. Carlsson, J. Engel, Eds. (North-Holland, Amsterdam, 1975), vol. II, pp. 219–229.
23. D. Clark, S. Hjorth, A. Carlsson, *Eur. J. Pharmacol.* **106**, 185 (1984).
24. _____, *J. Neural Transm.* **62**, 171 (1985).
25. C. A. Tamminga *et al.*, *J. Neural Transm.* [*Gen. Sect.*] **88**, 165 (1992).
26. A. C. Lahti *et al.*, *Biol. Psychiatry* **43**, 2 (1998).
27. W. H. Carson, M. Ali, G. Dunbar, G. Ingenito, A. R. Saha, *Schizophr. Res.* **49** (suppl.), 231 (2001).
28. K. Svensson *et al.*, *J. Neural Transm.* **65**, 1 (1986).
29. C. Sonesson *et al.*, *J. Med. Chem.* **36**, 3188 (1993).
30. J. Tedroff *et al.*, *Synapse* **28**, 280 (1998).
31. A. Carlsson, *Neuropsychopharmacology* **1**, 179 (1988).
32. M. Carlsson, A. Carlsson, *J. Neural Transm.* **75**, 221 (1989).
33. _____, *Trends Neurosci.* **13**, 272 (1990).
34. B. G. Bunney, W. E. Bunney Jr., A. Carlsson, in *Psychopharmacology: The Fourth Generation of Progress*, F. E. Bloom, D. J. Kupfer, Eds. (Raven, New York, 1995), pp. 1205–1214.
35. A. Svensson, M. L. Carlsson, A. Carlsson, *J. Neural Transm.* [*Gen. Sect.*] **101**, 127 (1995).
36. A. Carlsson, L. O. Hansson, N. Waters, M. L. Carlsson, *Life Sci.* **61**, 75 (1997).
37. P. Martin, N. Waters, C. J. Schmidt, A. Carlsson, M. L. Carlsson *J. Neural Transm.* **105**, 365 (1998).
38. A. Carlsson, L. O. Hansson, N. Waters, M. L. Carlsson, *Brit. J. Psychiatry* **174** (suppl.), 37 (1999).
39. M. L. Carlsson *et al.*, *J. Neural Transm.* **106**, 123 (1999).
40. S. G. Potkin *et al.*, *Schizophr. Res.* **49** (suppl.), 242 (2001).
41. A. Carlsson, *Psychiatr. Neurol.* **140**, 220 (1960).
42. L. S. Seiden, A. Carlsson, *Psychopharacologia* **5**, 178 (1964).
43. Å. Bertler, *Acta Physiol. Scand.* **51**, 75 (1961).
44. A. Carlsson, in *Mechanisms of Release of Biogenic Amines*, U. S. von Euler, S. Rosell, B. Uvnäs, Eds. (Pergamon, Oxford, 1966), pp. 331–346.

REVIEW: NEUROSCIENCE

The Neurobiology of Slow Synaptic Transmission

Paul Greengard

Nerve cells communicate with each other through two mechanisms, referred to as fast and slow synaptic transmission. Fast-acting neurotransmitters, e.g., glutamate (excitatory) and γ-aminobutyric acid (GABA) (inhibitory), achieve effects on their target cells within one millisecond by virtue of opening ligand-operated ion channels. In contrast, all of the effects of the biogenic amine and peptide neurotransmitters, as well as many of the effects of glutamate and GABA, are achieved over hundreds of milliseconds to minutes by slow synaptic transmission. This latter process is mediated through an enormously more complicated sequence of biochemical steps, involving second messengers, protein kinases, and protein phosphatases. Slow-acting neurotransmitters control the efficacy of fast synaptic transmission by regulating the efficiency of neurotransmitter release from presynaptic terminals and by regulating the efficiency with which fast-acting neurotransmitters produce their effects on postsynaptic receptors.

There are about 100 billion nerve cells in the brain, and on average each of these cells communicates directly with 1000 others. A vigorous debate went on from the 1930s through the 1960s as to whether communication across the synapses between nerve cells was electrical or chemical in nature. The electrical school of thought held that the nerve impulse or action potential was propagated along the axon to the nerve ending, changed the electrical field across the plasma membrane of the postsynaptic cell, and thereby produced a physiological response. The chemical school believed that when the action potential came down the axon to the nerve terminal, it caused the fusion of neurotransmitter-containing vesicles with the presynaptic plasma membrane, releasing the neurotransmitter, which then diffused across the synaptic cleft and, through activation of a (hypothetical) receptor, produced a physiological response. The chemical school won this debate: over 99% of all synapses in the brain use chemical transmission. On the basis of those earlier studies, I became interested in the biochemical mechanisms by which neurotransmitters, through activation of their receptors, produce their physiological effects within their postsynaptic, target nerve cells.

We know today that there are two categories of chemical transmission between nerve cells, which we refer to as fast and slow synaptic transmission. About half of the fast synapses in the brain are excitatory, and most of these fast excitatory synapses use gluta-

Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA. E-mail: greengd@mail.rockefeller.edu

mate as their neurotransmitter. The other half of the fast synapses are inhibitory, and most of these fast inhibitory synapses use GABA as their neurotransmitter. Synaptic transmission at fast synapses occurs in less than 1/1000 of a second and is attributable to the ability of the fast-acting neurotransmitters to open ligand-operated ion channels present in the plasma membrane of the postsynaptic cells. In fast excitatory transmission, glutamate binds to a receptor, causing a change in the conformation of the receptor, which allows positively charged sodium ions to rush into the cell and causes a depolarizing (that is, excitatory) signal to be generated in the target cell. In fast inhibitory transmission, GABA binds to its receptor, causing a change in the conformation of the receptor, which allows negatively charged chloride ions to permeate the cell and causes a hyperpolarizing (that is, inhibitory) signal to be generated in the target cell.

The second type of communication between nerve cells, slow synaptic transmission, occurs over periods of hundreds of milliseconds to minutes, and is enormously more complex than fast synaptic transmission. At least 100 compounds, which can be grouped into three chemical classes, namely biogenic amines, peptides, and amino acids, are now believed to serve as neurotransmitters in the brain. The vast majority of these putative neurotransmitters appear to work through slow synaptic transmission. Thus, it seems likely that all of the biogenic amines and all of the peptide neurotransmitters produce their effects on their target cells through slow synaptic transmission. In addition, even the fast acting neurotransmitters, including the amino acids glutamate and GABA, produce many of their effects through slow synaptic transmission pathways.

The work in my laboratory on the molecular basis of slow synaptic transmission was inspired by studies carried out by Earl W. Sutherland (*1*) and Edwin G. Krebs (*2*). Sutherland and Krebs were interested in understanding how the hormones glucagon and adrenaline break down glycogen to glucose in liver and muscle cells. Sutherland and his colleagues found that these hormones stimulated the formation of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP) by virtue of activating a class of enzymes termed hormone-sensitive adenylyl cyclases. Sutherland then showed that cAMP could mimic the hormones, causing the breakdown of glycogen to glucose. Krebs and his colleagues subsequently showed that cAMP caused the breakdown of glycogen to glucose by activating an enzyme that they called cAMP-dependent protein kinase (PKA). Protein kinases catalyze the reaction:

ATP + substrate
→ ADP + phosphosubstrate

Krebs further showed that one substrate for PKA, when phosphorylated, was itself an enzyme that caused the breakdown of glycogen to glucose. The action of protein kinases is reversed by means of an enzyme called a protein phosphatase

Phosphosubstrate → substrate
+ inorganic phosphate

When my colleagues and I started our work on the molecular basis of synaptic transmission, my hypothesis was that the same signaling machinery used by the endocrine system to break down glycogen to glucose might be used for communication between nerve cells. A concern with this concept was that hormones travel distances of up to 2 m between the sending cell and the receiving cell, whereas the distance across a synapse is roughly 1 millionth of a centimeter. Nevertheless, we decided to test this hypothesis. We searched in the brain for signaling enzymes analogous to those in liver and muscle. We found a family of adenylyl cyclases, analogous to the hormone-sensitive adenylyl cyclases described by Sutherland, which converted ATP to cAMP in the presence of neurotransmitters. The first of these, found by John W. Kebabian, was a dopamine-sensitive adenylyl cyclase: in the presence of dopamine, this membrane-bound enzyme stimulated formation of cAMP. Moreover, the data indicated that this enzyme might play a role in synaptic transmission (*3*).

At about the same time, Eishichi Miyamoto and J. F. Kuo demonstrated PKA activity in the brain (*4*). The concentration of this enzyme was enormously higher in brain than in liver. Even more intriguing was the fact that the enzyme was concentrated in the synaptic region of nerve cells. These data were consistent with a possible role for PKA in synaptic transmission. Soon thereafter, Kuo found a second, distinct class of regulated protein kinase, which we named cyclic guanosine monophosphate (cGMP)–dependent protein kinase (PKG) and which was activated selectively by cGMP rather than cAMP (*5*). PKG was present both in brain and in non-neural tissues. Subsequently, Howard Schulman discovered a third group of regulated protein kinases, which were stimulated by calcium in the presence of an unidentified endogenous heat-stable protein, later shown to be the calcium effector protein calmodulin (*6*).

The discovery in the nervous system of several neurotransmitter-sensitive enzymes that made cAMP and of several second messenger–dependent protein kinases strengthened our belief that second messengers and protein kinases might be involved in signaling in the brain. This idea was supported by the discovery of a large number of brain-specific substrate proteins for these protein kinases. Thus, S. Ivar Walaas and Angus C. Nairn found more than 100 substrate proteins for protein kinases that were highly enriched in or exclusively localized to the brain, some of which were present in very high concentrations (*7*, *8*). In the ensuing years, we showed that injections of various second messengers, protein kinases, and protein phosphatases, as well as activators, inhibitors and substrates of these enzymes, were able to either mimic or antagonize the ability of neurotransmitters to produce physiological responses in nerve cells, such as changes in ligand-gated ion channels, voltage-gated ion channels, ion pumps, and transcription factors. Combined, these data have provided overwhelming evidence of a role for those signal transduction pathways in synaptic transmission (*9*).

Some of the principal signaling pathways involved in slow synaptic transmission are shown in Fig. 1. In various studies (*10*–*15*), we demonstrated conclusively that the efficacy of neurotransmitter release from the presynaptic terminal, in response to the nerve impulse, is regulated by protein phosphorylation and dephosphorylation, but the details lie outside the scope of this presentation. Rather, I am going to concentrate on how slow-acting neurotransmitters, through activation of their receptors, produce appropriate physiological responses in their postsynaptic target cells.

Slow-acting neurotransmitters, upon binding to their receptors, change the level of a second messenger (for example, cAMP, cGMP, calcium, or diacylglycerol). These second messenger molecules, in turn, activate distinct classes of protein kinases. The activated protein kinases phosphorylate and thereby change the properties of substrate proteins, which serve as downstream physiological effectors. The substrate proteins of the nervous system can be divided into various classes, four of which are shown in Fig. 1. One class is the receptors for neurotransmitters, both fast- and slow-acting. These protein kinases also phosphorylate various voltage-gated sodium, potassium, and calcium ion channels. Another class of substrate proteins is the ion pumps, which restore ionic equilibrium after a burst of neuronal activity. Still another class is the transcription factors present in the cell nucleus, which control new protein synthesis, required for long-term changes in the nerve cell in response to activity and are likely an important component of the molecular basis of learning and memory, a field pioneered by Eric Kandel (*16*).

Although slow synaptic transmission, as shown in Fig. 1, is now part of the scientific dogma, it was greeted initially with enormous skepticism, and at times down-right hostility, by the scientific community. In retrospect, there were two major reasons for this oppo-

sition. First, when we started this work, neuroscience was not a clearly defined field. There were neuroanatomists and psychologists who studied the nervous system, but, at that time, those were essentially descriptive pursuits. In addition, there were two types of experimental scientists studying the brain. There were the biophysicists, who believed that everything important about the brain could be explained in terms of electrical signaling and that the function of biochemical activity in the brain was strictly to nourish nerve cells. And then there were the biochemists, who would happily throw a brain into a homogenizer, with as much abandon as they would a liver, and look for enzymes or lipids. But these biochemists were rarely interested in brain function. And so these two groups rarely spoke to each other, which is just as well because when they did, they did not have nice things to say. So, there was almost no one working on the biochemical basis of how nerve cells function. A second reason for the skepticism was more substantive: it was not immediately obvious how the relatively slow enzymatic reactions that we were studying, protein phosphorylation and dephosphorylation, could be involved in fast synaptic transmission, which, as Llinas has shown (*17*), occurs in less than 1/1000 of a second. As it turned out, these slow signaling pathways do not mediate fast synaptic transmission. Rather, they modulate fast synaptic transmission, and they do so in two major ways: (i) by regulating the phosphorylation state of synapsins and other key proteins in the presynaptic terminal, thus modulating the efficacy of neurotransmitter release (the amount of neurotransmitter released from the nerve terminal in response to an action potential), and (ii) by regulating the state of phosphorylation of neurotransmitter receptors present in the plasma membrane of the postsynaptic cell, thus modulating the responsivity of these receptors to the released neurotransmitter (that is, the magnitude of the electrophysiological response to a molecule of neurotransmitter).

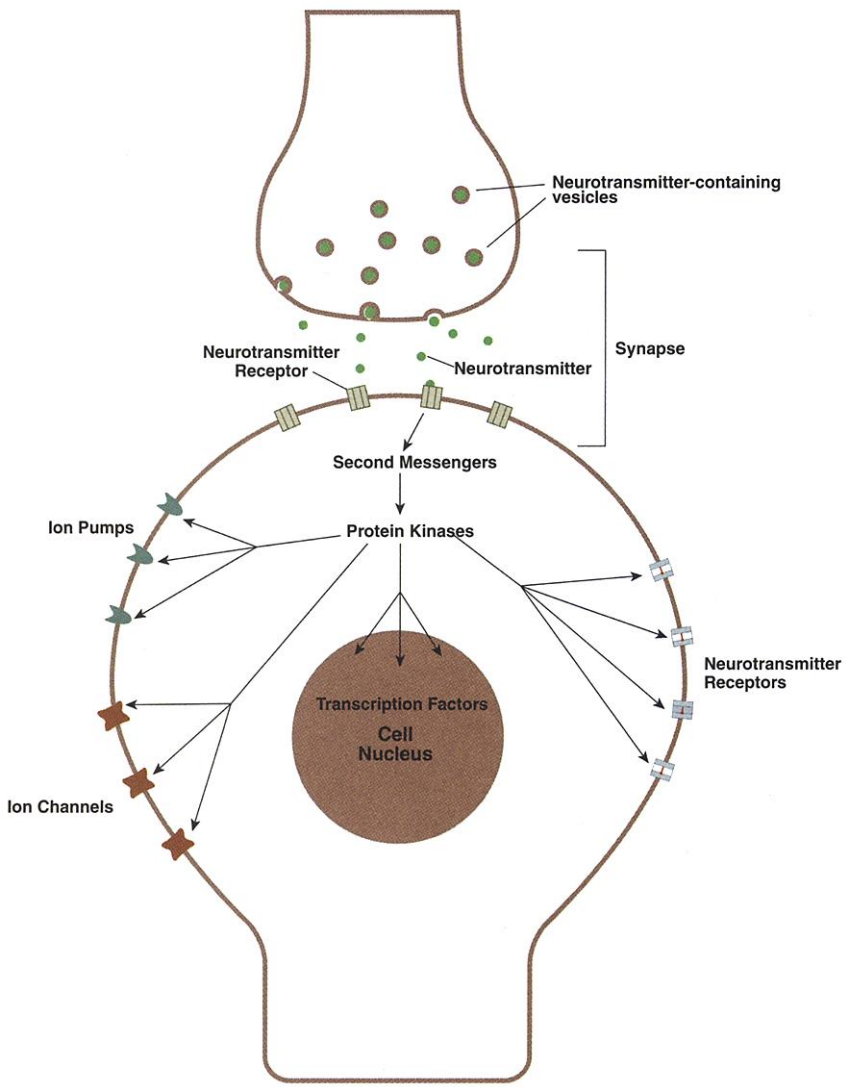

Fig. 1. Some of the signaling pathways of slow synaptic transmission.

The slow signaling pathways that have been studied to date obey similar principles. The slow-acting neurotransmitter that we have studied most intensively is dopamine. There were several reasons for focusing on this system. First, there was the pioneering work of Arvid Carlsson (*18*), his colleagues, and those who followed in his footsteps, showing that four major and several minor neurological and psychiatric diseases are associated with abnormalities in the dopamine signaling pathway. The four major diseases are Parkinsonism, schizophrenia, Attention Deficit Hyperactivity Disorder (ADHD), and drug abuse. Parkinsonism is associated with the death of dopamine-producing nerve cells and is treated by giving levodopa, a precursor of dopamine. Most drugs currently used for the treatment of schizophrenia block a subclass of dopamine receptors. ADHD is treated with Ritalin, which works in large part by stimulating dopamine release. Virtually all drugs of abuse cause perturbations of dopamine signaling. In addition, the neurostriatum, a major target for dopaminergic innervation, is relatively large and homogenous and, thus, fairly readily permits both electrophysiological and biochemical studies. And the simple circuitry of the basal ganglia, compared with the circuitry of the cortex, makes the analysis of signaling systems much more manageable. Although much of our research has concentrated on dopamine pathways, the principles elucidated in those pathways appear to be applicable to all slow synaptic pathways in the brain.

In our studies of dopamine signaling, we were very fortunate to discover a molecule that we named DARPP-32, an acronym for dopamine and cAMP-regulated phosphoprotein (molecular weight = 32 kD). Dopamine, by activation of a subclass of dopamine receptors, causes an increase in the level of cAMP, the activation of PKA, and the phosphorylation of threonine-34 of DARPP-32. DARPP-32 is necessary to mediate the actions of dopamine and has served as a Rosetta Stone for understanding the mechanism of action of dopamine and its interactions with other neurotransmitters, therapeutic drugs, and drugs of abuse. DARPP-32 was discovered by S. Ivar Walaas and Dana W. Aswad while they were searching for region-specific protein kinase substrates in the brain (*19*). In the ensuing years, DARPP-32 has been extensively characterized with respect to its biochemical properties and physiological roles (*19–45*).

DARPP-32 is highly concentrated in the neostriatum (the caudate and the putamen) and the nucleus accumbens. The neostriatal neurons that contain DARPP-32 are the only efferent pathway for conveying information out of this major brain region. These neurons must integrate information entering the neo-

striatum from many other regions in the brain, and DARPP-32 plays a central role in this integration process. One major afferent pathway is composed of neurons that project from the cortex to the striatum and use glutamate to excite the DARPP-32–containing neurons. The excitability of the DARPP-32–containing neurons is modulated by dopaminergic neurons that project from the substantia nigra to the neostriatum. The regulation of the excitability of the DARPP-32–containing neurons has provided a useful model for studying the mechanisms by which slow synaptic transmission, as exemplified by dopamine, modulates fast synaptic transmission, as exemplified by glutamate.

There are three major classes of glutamate receptors, designated NMDA (*N*-methyl-D-aspartate), AMPA, and metabotropic, and two major classes of dopamine receptors, designated D1 and D2. The interactions between the dopamine and glutamate signaling pathways are complex and are modulated by many other neurotransmitters and their signaling pathways. DARPP-32 plays a central role in the interactions amongst those various complex signaling pathways (Fig. 2). All four mechanisms for regulating the phosphorylation of DARPP-32 on threonine-34 have been shown to exist, i.e., increases and decreases in phosphorylation and increases and decreases in dephosphorylation. Why has so much evolutionary machinery gone into regulating phosphorylation of DARPP-32?

The DARPP-32 sequence has been highly conserved in mammals. Rat DARPP-32 is a protein consisting of 205 amino acids. Threonine-34 of DARPP-32 is phosphorylated by PKA or PKG and dephosphorylated by protein phosphatase 2B (PP2B). Phosphorylation of DARPP-32 on threonine-34 profoundly changes its biological properties, converting it from an inactive molecule into a very potent inhibitor of protein phosphatase 1 (PP1), with an IC_{50} (median inhibitory concentration) and K_i (inhibition constant) of about 10^{-9} M (*39*). Because the concentration of DARPP-32 in medium spiny neurons is greater than 10^{-5} M, a small burst of activity in dopaminergic neurons would result in significant phosphorylation of DARPP-32 and inhibition of PP1. Protein phosphatase 1 has a very broad substrate specificity and controls the state of phosphorylation and activity of numerous physiologically important substrates, including neurotransmitter receptors, voltage-gated ion channels, ion pumps, and transcription factors. As a result, neurotransmitters that increase or decrease phospho–threonine-34 of DARPP-32 inhibit or activate, respectively, protein phosphatase 1, and thereby increase or decrease the state of phosphorylation and activity of a large array of downstream physiological effectors.

The physiological significance of the DARPP-32–PP1 cascade has been demonstrated in two types of experiments. In one type, we injected protein kinases, protein phosphatases, or inhibitors or activators thereof into medium spiny neurons and obtained physiological responses consistent with the scheme shown in Fig. 2. The other type of study involved analysis of mice with targeted deletion of the DARPP-32 gene. Allen Fienberg engineered this "knockout" mouse and, in a multi-institutional collaboration, found that all behavioral, physiological, biochemical, pharmacological, and toxicological responses to dopamine, the psychostimulant drugs of abuse, and antischizophrenic drugs seen in normal mice were either greatly diminished or abolished in DARPP-32 knockout mice (*40*).

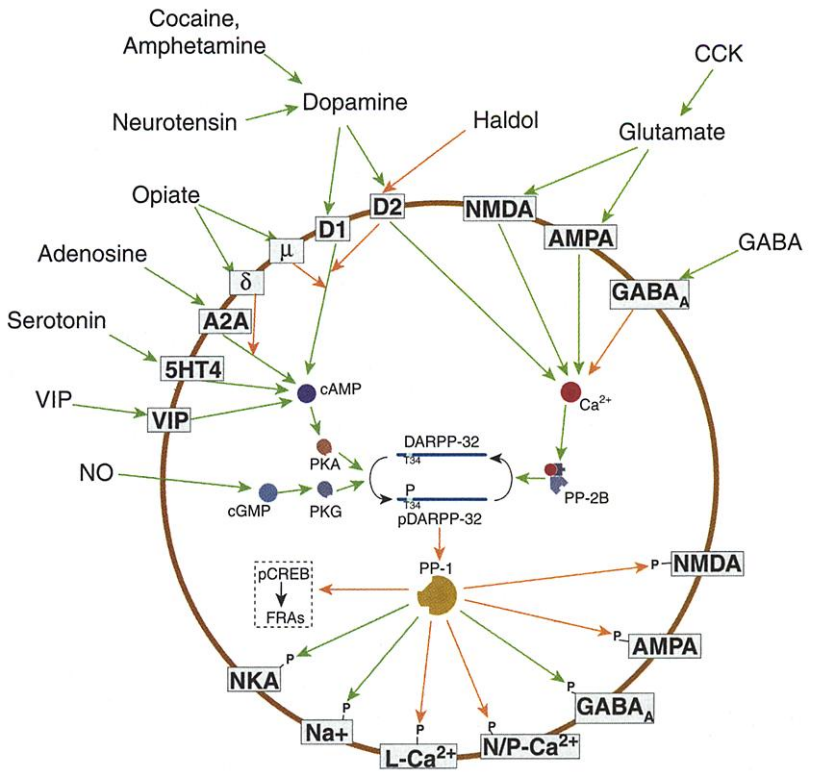

Fig. 2. Signaling pathways in the neostriatum. Activation by dopamine of the D1 subclass of dopamine receptors stimulates the phosphorylation of DARPP-32 at Thr-34. This is achieved through a pathway involving the activation of adenylyl cyclase, the formation of cAMP, and the activation of PKA. Activation by dopamine of the D2 subclass of dopamine receptors causes the dephosphorylation of DARPP-32 through two synergistic mechanisms: D2 receptor activation (i) prevents the D1 receptor-induced increase in cyclic AMP formation, and (ii) raises intracellular calcium, which activates a calcium-dependent protein phosphatase, namely PP2B, calcium/calmodulin-dependent protein phosphatase, or calcineurin. Activated PP2B dephosphorylatès DARPP-32 at Thr-34. Glutamate acts as both a fast-acting and slow-acting neurotransmitter. Activation by glutamate of AMPA receptors causes a rapid response through influx of sodium ions, depolarization of the membrane, and firing of an action potential. Slow synaptic transmission, in response to glutamate, results in part from activation of the AMPA and NMDA subclasses of glutamate receptor, which increases intracellular calcium and the activity of PP2B, and causes the dephosphorylation of DARPP-32 on Thr-34. All other neurotransmitters that have been shown to act directly to alter the physiology of dopaminoceptive neurons also alter the phosphorylation state of DARPP-32 on Thr-34 through the indicated pathways. Neurotransmitters that act indirectly to affect the physiology of these dopaminoceptive neurons also regulate DARPP-32 phosphorylation: e.g., neurotensin, through stimulating the release of dopamine, increases DARPP-32 phosphorylation; conversely cholecystokinin (CCK), by stimulating the release of glutamate, decreases DARPP-32 phosphorylation. Antischizophrenic drugs and drugs of abuse, all of which affect the physiology of these neurons, also regulate the state of phosphorylation of DARPP-32 on Thr-34. For example, the antischizophrenic drug Haldol, which blocks the activation by dopamine of the D2 subclass of dopamine receptor, increases DARPP-32 phosphorylation. Agonists for the mu and delta subclasses of opiate receptors block D1 and A2A receptor–mediated increases in cAMP, respectively, and the resultant increases in DARPP-32 phosphorylation. Cocaine and amphetamine, through increasing extracellular dopamine levels, increase DARPP-32 phosphorylation. Marijuana, nicotine, alcohol, and LSD, all of which affect the physiology of the dopaminoceptive neurons, also regulate DARPP-32 phosphorylation. Lastly, all drugs of abuse have greatly reduced biological effects in animals with targeted deletion of the DARPP-32 gene. 5HT4, 5 hydroxytryptophan (serotonin) receptor 4; NKA, Na^+,K^+ ATPase; VIP, vasoactive intestinal peptide; L- and N/P-Ca^{2+}, L type and N/P type calcium channels.

The fast excitatory glutamate receptors, AMPA and NMDA, regulate and are regulated by the DARPP-32–PP1 cascade (Fig. 2). From this scheme, one would predict that PP1 would be localized in the vicinity of these glutamate receptors. Immunocytochemical experiments, with antibodies prepared against recombinant PP1, enabled us to localize this phosphatase in neostriatal cells. PP1 was enriched in the spines of the dendrites of medium spiny neurons (*46*). To determine the basis for the enrichment of PP1 in spines, Patrick Allen used yeast–two hybrid technology to search for a PP1-targeting protein. He found a molecule, which he named spinophilin, with the properties of such a targeting protein. Spinophilin is localized almost exclusively in dendritic spine heads at excitatory synapses (*41*), precisely where the AMPA and NMDA receptors are concentrated.

Spinophilin, through control of PP-1, regulates the conductance properties of AMPA (Fig. 3) and NMDA receptors. In its dephosphorylated form, the AMPA receptor is relatively insensitive to activation by glutamate. Its sensitivity to glutamate is greatly enhanced upon phosphorylation by cAMP-dependent protein kinase (*47*, *48*). In the absence of dopamine, the AMPA receptor is kept in a low-conductance state by PP1. Dopamine, through activation of cAMP-dependent protein kinase, causes an increase in the state of phosphorylation of the GluR1 subunit of the AMPA receptor, and this effect is greatly attenuated in the neostriatum of mice lacking the DARPP-32 gene. The activation of PKA in response to dopamine increases the state of phosphorylation of the AMPA receptor by a synergistic mechanism involving direct phosphorylation of the receptor as well as phosphorylation of threonine-34 on DARPP-32, which results in inhibition of PP1-catalyzed dephosphorylation of the receptor.

Physiological support for the scheme shown in Fig. 3 includes evidence that the dephosphorylation and the associated loss of responsivity of AMPA receptors to activation by glutamate can be prevented by incubation of cells either with a D1 receptor agonist, or with the PP1 inhibitor okadaic acid, but not with an inactive analog of okadaic acid (*42*). In addition, injection of a peptide corresponding to residues 6–38 of phospho–DARPP-32, which retains the ability to inhibit PP1, but not residues 6–38 of dephospho–DARPP-32, which does not inhibit PP1, prevents loss of AMPA receptor responsivity. Injection of a spinophilin-based peptide, which prevents tethering of PP1 to spinophilin, also prevents AMPA receptor inactivation; a single point mutation abolishes both effects of this peptide. Spinophilin regulates the NMDA receptor in a manner parallel to that by which it regulates the AMPA receptor (*42*). Spinophilin does not regulate the fast inhibitory GABA-A receptor. Thus, spinophilin appears to control fast excitatory, but not fast inhibitory, synaptic transmission. Lastly, spinophilin itself is a substrate for PKA, and dopamine-induced PKA-mediated phosphorylation of spinophilin appears to play a role in the complex mechanism by which dopamine controls the efficacy of synaptic transmission at fast excitatory synapses (*49*).

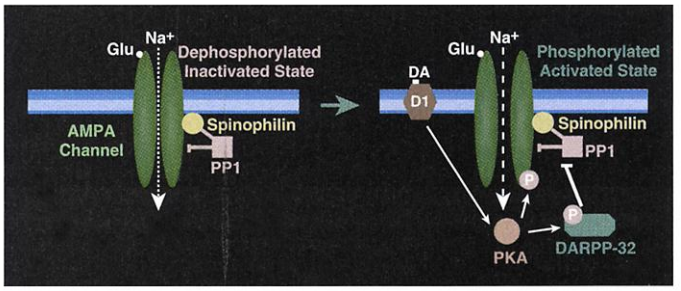

Fig. 3. Regulation of AMPA-type glutamate receptors by DARPP-32 and spinophilin. Model illustrates how regulation of PP1 could account for the ability of DARPP-32 and spinophilin to control the phosphorylation state and conductance properties of AMPA channels. (Left) Spinophilin, by binding to an unidentified intermediate protein (not shown), localizes PP-1 in the vicinity of the AMPA channel. Under basal conditions, the PP1–spinophilin complex maintains the AMPA channel in a dephosphorylated state in which it is relatively insensitive to its neurotransmitter, glutamate. (Right) After D1 receptor stimulation, AMPA channel phosphorylation is increased due both to direct PKA phosphorylation and to PKA/phospho–DARPP-32–mediated inhibition of PP1. This synergistic increase in phosphorylation converts the AMPA channel into a form that is more responsive to glutamate. The kinase anchoring protein (AKAP) believed to localize PKA in the vicinity of the AMPA receptor (*50*) is not shown. [Modified from Greengard *et al.* (*9*)]

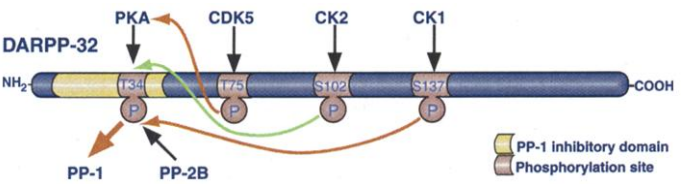

Fig. 4. Multiple phosphorylation sites on DARPP-32. DARPP-32 is phosphorylated at Thr-34 by PKA (and PKG, not shown) and dephosphorylated by PP2B. Phosphorylation at Thr-34 converts DARPP-32 into a potent inhibitor of PP1. DARPP-32 is phosphorylated on Ser-137 by casein kinase I. Phosphorylation at Ser-137 converts DARPP-32 into a poorer substrate for PP2B-catalyzed dephosphorylation of Thr-34 without affecting phosphorylation of Thr-34 by PKA or PKG and without affecting the ability of PP2B to dephosphorylate other substrates (*44*). DARPP-32 is phosphorylated on Ser-102 by casein kinase II. Phosphorylation at Ser-102 converts DARPP-32 into a better substrate for phosphorylation by PKA, without affecting its phosphorylation by PKG or its dephosphorylation by PP2B and without affecting the ability of PKA to phosphorylate other substrates (*43*). Thus, the effects of phospho–Ser-137 DARPP-32 and phospho–Ser-102 DARPP-32 are substrate-directed, not enzyme-directed. DARPP-32 is phosphorylated on Thr-75 by CDK5. Phosphorylation at Thr-75 converts DARPP-32 into an inhibitor of PKA, reducing its ability to phosphorylate any substrate, including DARPP-32 at Thr-34 (*45*). Thus the effect of phospho–Thr-75 DARPP-32 is enzyme-directed, not substrate-directed. Because dopamine increases and glutamate decreases phosphorylation of DARPP-32 at Thr-34, signaling mediated through phosphorylation or dephosphorylation of Ser-137, Ser-102, and Thr-75 alters the balance between dopaminergic and glutamatergic signaling. Specifically, the casein kinase 1–Ser-137 and casein kinase 2–Ser-102 pathways are prodopaminergic or antiglutamatergic, whereas the CDK5–Thr-75 pathway is antidopaminergic or proglutamatergic. Phospho–Ser-137 is preferentially dephosphorylated by PP2C (not shown). The NH_2-terminal domain of DARPP-32, which binds to PP1, is shown in yellow. Red arrows indicate inhibition; green arrow indicates stimulation. [Modified from Greengard *et al.* (*9*)]

Balancing Excitation and Inhibition

The percentage of DARPP-32 molecules in the phosphorylated state and the degree of inhibition of PP1 reflect a balance between the rates of phosphorylation and dephosphorylation of threonine-34 (Fig. 2). The effectiveness of PKA and PP2B in regulating threonine-34 phosphorylation and dephosphorylation is itself regulated by other protein kinases and protein phosphatases. We now know of three sites on DARPP-32 in addition to threonine-34 that are phosphorylated in response to activity in other signaling pathways. Each of these three pathways modulates the dopamine/D1 receptor/cAMP/PKA/phosphothreonine-34–DARPP-32/PP1 cascade (Fig. 4) (*43*–*45*). DARPP-32 can be either a protein phosphatase inhibitor or a protein kinase inhibitor, depending on whether threonine-34 or threonine-75 is phosphorylated. Although DARPP-32 is the only bifunctional molecule of this type found to date, it seems likely that other proteins will be found that can serve either as a protein kinase inhibitor or a protein phosphatase inhibitor, depending on the residue phosphorylated.

A major mechanism by which dopamine and glutamate produce opposing physiological effects appears to involve a positive feed-

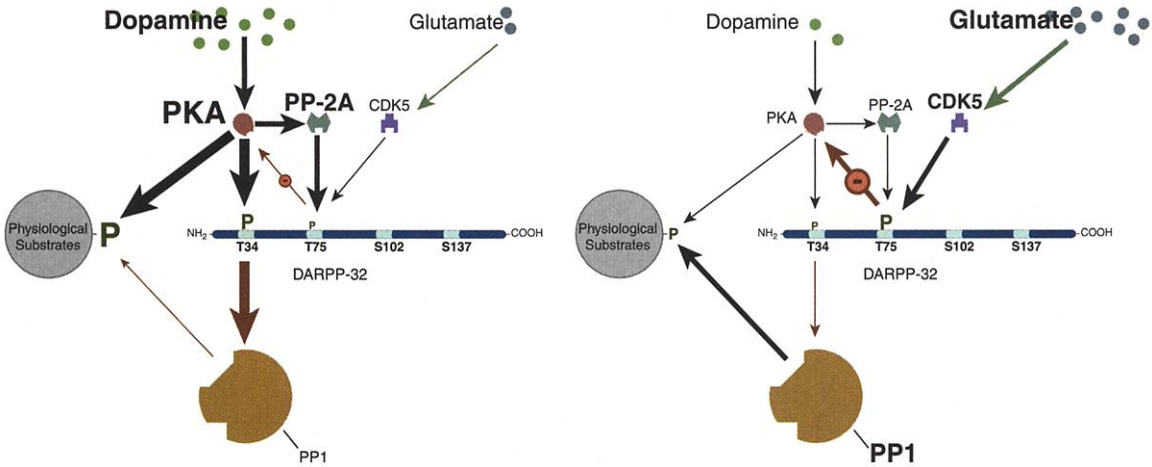

Fig. 5. Model illustrating some of the signaling pathways involved in mediating opposing biological actions of the neurotransmitters dopamine and glutamate. Larger letters and arrows indicate higher levels and smaller letters and arrows indicate lower levels of dopamine and glutamate; of the activity of the enzymes PKA, PP2A, CDK5, and PP1; of the PKA inhibitor phospho–Thr-75 DARPP-32; and of the PP1 inhibitor phospho–Thr-34 DARPP-32.

back loop that amplifies their mutually antagonistic actions (Fig. 5). This positive feedback loop has three components: PKA, protein phosphatase 2A (PP2A), and threonine-75 of DARPP-32. In resting animals, threonine-75 is very highly phosphorylated, whereas threonine-34 is only slightly phosphorylated. Tonic activity of the glutamate-CDK5 (cyclin-dependent kinase 5) pathway is probably responsible for keeping threonine-75 phosphorylated, and thereby inhibiting PKA. Dopamine, by activating D1 receptors, increases the activity of PKA, leading to phosphorylation of key physiological substrate proteins. Increased PKA activity also increases phosphorylation of threonine-34 on DARPP-32, inhibiting PP1, and thereby decreasing the dephosphorylation of these physiological substrates. PKA also activates PP2A, which dephosphorylates threonine-75, which thereby removes the inhibition of PKA. Thus, dopamine causes both an increased activation and a decreased inhibition of PKA. Conversely, glutamate activates CDK5 and, by phosphorylating threonine-75, inhibits PKA, reducing the activity of PP2A, and the resultant dephosphorylation of threonine-75. Through this mechanism, glutamate causes both an increased phosphorylation and a decreased dephosphorylation of threonine-75. Thus the PKA/PP2A/threonine-75 DARPP-32 triad amplifies either the effects of dopamine or the effects of glutamate, whichever is the more dominant neurotransmitter at any given time.

Concluding Remarks

The schemes shown in Figs. 2, 4, and 5 summarize only a portion of the highly complex pathways that we now know to be involved in signal transduction in dopaminoceptive neurons. The differences between the lack of complexity of fast synaptic transmission, in which there is a single ligand-operated ion channel, and the enormously complicated pathways underlying slow synaptic transmission, only part of which we have elucidated, seem amazing. However, when one thinks of fast synaptic transmission as being the hardware of the brain, and slow synaptic transmission as being the software that controls fast transmission, the molecular basis by which nerve cells communicate with each other makes more sense.

The elucidation of the principles underlying slow synaptic transmission and the discovery of numerous components of the underlying intracellular signaling pathways have provided a number of newly found therapeutic targets for the treatment of neurological and psychiatric illnesses associated with abnormalities of dopamine signaling. For instance, levodopa is effective in most patients with Parkinson's disease. Levodopa is converted to dopamine, which then activates dopamine receptors and alleviates the disease symptoms. Unfortunately, within a short time, many Parkinsonian patients become refractory to levodopa treatment, probably a result in part of down-regulation of dopamine receptors. It should be possible to develop various therapeutic substances for Parkinsonism that activate or inhibit various intracellular dopamine signaling components without the refractoriness associated with levodopa treatment. Drugs that target intracellular constituents of dopaminoceptive neurons may also be useful for treatment of other neurological and psychiatric disorders associated with dopamine signaling abnormalities. Lastly, because the various slow acting neurotransmitters work by similar principles, it should be possible to find drugs working intracellularly for the treatment of diseases affecting parts of the brain where signaling pathways other than dopamine are used.

References and Notes

1. E. W. Sutherland, in *Les Prix Nobel 1971* (Imprimerie Royale, P. A. Norstedt & Söner, Stockholm, Sweden, 1972), pp. 240–257.
2. E. G. Krebs, in *Les Prix Nobel 1992* (Almqvist & Wiksell International, Stockholm, Sweden, 1992), pp. 98–115.
3. J. W. Kebabian, P. Greengard, *Science* **174**, 1346 (1971).
4. E. Miyamoto, J. F. Kuo, P. Greengard, *Science* **165**, 63 (1969).
5. J. F. Kuo, P. Greengard, *J. Biol. Chem.* **245**, 2493 (1970).
6. H. Schulman, P. Greengard, *Nature* **271**, 478 (1978).
7. S. I. Walaas, A. C. Nairn, P. Greengard, *J. Neurosci.* **3**, 291 (1983).
8. ———, *J. Neurosci.* **3**, 302 (1983).
9. P. Greengard, P. B. Allen, A. C. Nairn, *Neuron* **23**, 435 (1999).
10. R. Llinas, T. L. McGuinness, C. S. Leonard, M. Sugimori, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 3035 (1985).
11. R. Llinàs, J. A. Gruner, M. Sugimori, T. L. McGuinness, P. Greengard, *J. Physiol.* **436**, 257 (1991).
12. P. Greengard, F. Valtorta, A. J. Czernik, F. Benfenati, *Science* **259**, 780 (1993).
13. P. DeCamilli, F. Benfenati, F. Valtorta, P. Greengard, *Annu. Rev. Cell Biol.* **6**, 433 (1990).
14. V. F. Castellucci *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7492 (1980).
15. V. F. Castellucci, A. Nairn, P. Greengard, J. H. Schwartz, E. R. Kandel, *J. Neurosci.* **2**, 1673 (1982).
16. E. R. Kandel, in *Les Prix Nobel 2000* (Almqvist & Wiksell International, Stockhom, Sweden, 2000), pp. 326–373.
17. R. Llinas, I. Z. Steinberg, K. Walton, *Biophys. J.* **33**, 323 (1981).
18. A. Carlsson, in *Les Prix Nobel 2000* (Almqvist & Wiksell International, Stockholm, Sweden, 2000), pp. 237–256.
19. S. I. Walaas, D. W. Aswad, P. Greengard, *Nature* **301**, 69 (1983).
20. A. C. Nairn, J. A. Detre, J. E. Casnellie, P. Greengard, *Nature* **299**, 734 (1982).
21. E. J. Nestler, P. Greengard, *Protein Phosphorylation in the Nervous System* (Wiley, New York, 1984).
22. C. C. Ouimet, P. E. Miller, H. C. Hemmings Jr., S. I. Walaas, P. Greengard, *J. Neurosci.* **4**, 111 (1984)
23. J.-A. Girault, I. A. Shalaby, N. L. Rosen, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 7790 (1988).
24. C. C. Ouimet, H. C. Hemmings Jr., P. Greengard, in *Central D1 Dopamine Receptors*, M. Goldstein, I. Tabachnick, Eds. (Plenum, New York, 1988), vol. 235, pp. 1–17.
25. C. C. Ouimet, A. S. LaMantia, P. Goldman-Rakic, P. Rakic, P. Greengard, *J. Comp. Neurol.* **323**, 209 (1992).
26. G. L. Snyder *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 11277 (1993).
27. G. L. Snyder, G. Fisone, P. Greengard, *J. Neurochem.* **63**, 1766 (1994).

28. F. Desdouits, J. C. Siciliano, P. Greengard, J. A. Girault, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 2682 (1995).
29. A. Nishi, G. L. Snyder, P. Greengard, *J. Neurosci.* **17**, 8147 (1997).
30. G. L. Snyder, A. A. Fienberg, R. L. Huganir, P. Greengard, *J. Neurosci.* **18**, 10297 (1998)
31. P. Svenningsson *et al.*, *Neurosci.* **84**, 223 (1998).
32. A. Nishi, G. L. Snyder, A. C. Nairn, P. Greengard, *J. Neurochem.* **72**, 2015 (1999).
33. P. Svenningsson *et al.*, *J. Neurochem.* **75**, 248 (2000).
34. G. L. Snyder *et al.*, *J. Neurosci.* **20**, 4480 (2000).
35. A. A. Fienberg, P. Greengard, *Brain Res. Rev.* **31**, 313 (2000).
36. P. Svenningsson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 1856 (2000).
37. A. Nishi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 12840 (2000).
38. J. A. Bibb *et al.*, *Nature* **410**, 376 (2001).
39. H. C. Hemmings Jr., P. Greengard, H. Y. L. Tung, P. Cohen, *Nature* **310**, 503 (1984).
40. A. A. Fienberg *et al.*, *Science* **281**, 838 (1998).
41. P. B. Allen, C. C. Ouimet, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9956 (1997).
42. Z. Yan *et al.*, *Nature Neurosci.* **2**, 13 (1999).
43. J.-A. Girault, H. C. Hemmings Jr., K. R. Williams, A. C. Nairn, P. Greengard, *J. Biol. Chem.* **264**, 21748 (1989).
44. F. Desdouits, D. Cohen, A. C. Nairn, P. Greengard, J.-A. Girault, *J. Biol. Chem.* **270**, 8772 (1995).
45. J. A. Bibb *et al.*, *Nature* **402**(6762), 669 (1999).
46. E. F. da Cruz e Silva *et al.*, *J. Neurosci.* **15**(5), 3375 (1995).
47. P. Greengard, J. Jen, A. C. Nairn, C. F. Stevens, *Science* **253**, 1135 (1991).
48. L.-Y Wang, M. W. Salter, J. F. MacDonald, *Science* **253**, 1132 (1991).
49. L. Hsieh-Wilson *et al.*, unpublished data.
50. C. Rosenmund *et al.*, *Nature* **368**, 853 (1994).
51. The work summarized here reflects outstanding contributions from many highly gifted associates who have worked in our laboratory. I would particularly like to mention A. C. Nairn, who has been a close colleague and friend for more than 20 years. This work has also benefited enormously from collaborations with excellent scientists at several other universities. Our work on regulation of ion pumps was carried out in collaboration with A. Aperia at the Karolinska Institute. We continue to collaborate with R. L. Huganir, who was at The Rockefeller University, and is now at The Johns Hopkins University School of Medicine and with E. J. Nestler, who was at the Yale University School of Medicine and is now at the University of Texas Southwestern Medical Center. Much of our electrophysiological work has been done in collaboration with D. J. Surmeier at Northwestern University. The work of our research group has been very generously supported for over 30 years by the National Institutes of Health, including the National Institute of Mental Health, the National Institute on Drug Abuse, and the National Institute on Aging.

REVIEW: NEUROSCIENCE

The Molecular Biology of Memory Storage: A Dialogue Between Genes and Synapses

Eric R. Kandel*

One of the most remarkable aspects of an animal's behavior is the ability to modify that behavior by learning, an ability that reaches its highest form in human beings. For me, learning and memory have proven to be endlessly fascinating mental processes because they address one of the fundamental features of human activity: our ability to acquire new ideas from experience and to retain these ideas over time in memory. Moreover, unlike other mental processes such as thought, language, and consciousness, learning seemed from the outset to be readily accessible to cellular and molecular analysis. I, therefore, have been curious to know: What changes in the brain when we learn? And, once something is learned, how is that information retained in the brain? I have tried to address these questions through a reductionist approach that would allow me to investigate elementary forms of learning and memory at a cellular molecular level—as specific molecular activities within identified nerve cells.

I first became interested in the study of memory in 1950 as a result of my readings in psychoanalysis while still an undergraduate at Harvard College. Later, during medical training, I began to find the psychoanalytic approach limiting because it tended to treat the brain, the organ that generates behavior, as a black box. In the mid-1950s, while still in medical school, I began to appreciate that during my lifetime the black box of the brain would be opened and that the problems of memory storage, once the exclusive domain of psychologists and psychoanalysts, could be investigated with the methods of modern biology. As a result, my interest in memory shifted from a psychoanalytic to a biological approach. As a postdoctoral fellow at the National Institutes of Health (NIH) in Bethesda from 1957 to 1960, I focused on learning more about the biology of the brain and became interested in knowing how learning produces changes in the neural networks of the brain.

My purpose in translating questions about the psychology of learning into the empirical language of biology was not to replace the logic of psychology or psychoanalysis with the logic of cellular molecular biology, but to try to join these two disciplines and to contribute to a new synthesis that would combine the mentalistic psychology of memory storage with the biology of neuronal signaling. I hoped further that the biological analysis of memory might carry with it an extra bonus, that the study of memory storage might reveal new aspects of neuronal signaling. Indeed, this has proven true.

A Radical Reductionist Strategy to Learning and Memory

At first thought, someone interested in learning and memory might be tempted to tackle the problem in its most complex and interesting form. This was the approach that Alden Spencer and I took when we joined forces at NIH in 1958 to study the cellular properties of the hippocampus, the part of the mammalian brain thought to be most directly involved in aspects of complex memory (*1*). We initially asked, rather naïvely: Are the electrophysiological properties of the pyramidal cells of the hippocampus, which were thought to be the key hippocampal cells involved in memory storage, fundamentally different from other neurons in the brain? With study, it became clear to us that all nerve cells, including the pyramidal cells of the hippocampus, have similar signaling properties. Therefore, the intrinsic signaling properties of neurons would themselves not give us key insights into memory storage (*2*). The unique functions of the hippocampus had to arise not so much from the intrinsic properties of pyramidal neurons but from the pattern of functional interconnections of these cells, and how those interconnections are affected by learning. To tackle that problem we needed to know how sensory information about a learning task reaches the hippocampus and how information processed by the hippocampus influences behavioral output. This was a formidable challenge, since the hippocampus has a large number of neurons and an immense number of interconnections. It seemed unlikely that we would be able to work out in any reasonable period of time how the neural networks, in which the hippocampus was embedded, participate in behavior and how those networks are affected by learning.

To bring the power of modern biology to bear on the study of learning, it seemed necessary to take a very different approach—a radically reductionist approach. We needed

Howard Hughes Medical Institute, Center for Neurobiology and Behavior, College of Physicians and Surgeons of Columbia University, New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY 10032, USA. E-mail: erk5@columbia.edu

*This essay is adapted from the author's address to the Nobel Foundation, December 2000.