

Nobel Prize 2000 in Physiology or Medicine

Trackers of the Slow Synaptic Phenomena in the Brain

S K Sikdar

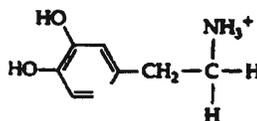


S K Sikdar is an associate professor at the Molecular Biophysics Unit, Indian Institute of Science, Bangalore. His laboratory specializes in the application of the electrophysiological technique of patch-clamp to understand how ion channel proteins are important in neuronal excitability function. The current research interest is in combined application of electrophysiological and imaging techniques to understand how neurons communicate with each other *in vitro*.

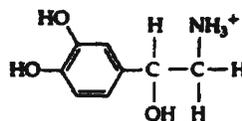
Our brain is made up of billions of neurons, which are special cells in the body. They are rather unique in two main aspects viz. they have diverse structures and they carry information in the form of electrical signals. The neurons communicate with each other at tiny junctions where opposing cellular elements meet to form the synapse, consisting of the presynaptic component which has the chemical called a neurotransmitter (*Figure 1*) stored in little bags called vesicles. Electrical activation of the presynaptic neuron reaching the nerve ending results in the fusion of the vesicle and thereby release of the neurotransmitter in the tiny gap between the pre- and post-synaptic nerve endings called the synaptic cleft. The neurotransmitter then acts on the receptors in the postsynaptic membrane, which may be channels themselves, or regulate channel opening through activation of intracellular messengers. In the former case the synaptic action is fast

BIOGENIC AMINES

Catecholamines

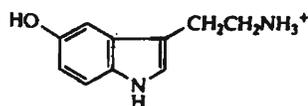


Dopamine



Norepinephrine (NE)
(Noradrenaline)

Indoleamine



5-Hydroxytryptamine
(Serotonin, 5-HT)

Figure 1. Chemical structures of certain biogenic amines functioning as neurotransmitters in the brain, related to the work of the Nobel laureates mentioned in the text.

and almost immediate, limited by the diffusion of the neurotransmitter to the receptor channel, while in the latter the response is slow.

The Nobel citation of the awards made for 2000 reads as follows “*The award has been given for studies on signal transduction in the nervous system*”. One of the key neurotransmitter molecule whose discovery was phenomenal and essentially made by Arvid Carlsson in the 1950s is dopamine. *Figure 2* shows the biochemical pathway for the synthesis of norepinephrine. Dopamine was thought to act as a precursor of norepinephrine, but not a neurotransmitter until the experiments of Arvid Carlsson proved otherwise. In one such experiment, Carlsson and his colleagues gave reserpine to experimental rabbits, a drug which was gaining use in the 1950s, which essentially depletes norepinephrine in the brain making the animals go into stupor. Upon injection of L-dopa, however the animals became active, and examination of the neurotransmitters in the brain, he found increased levels of

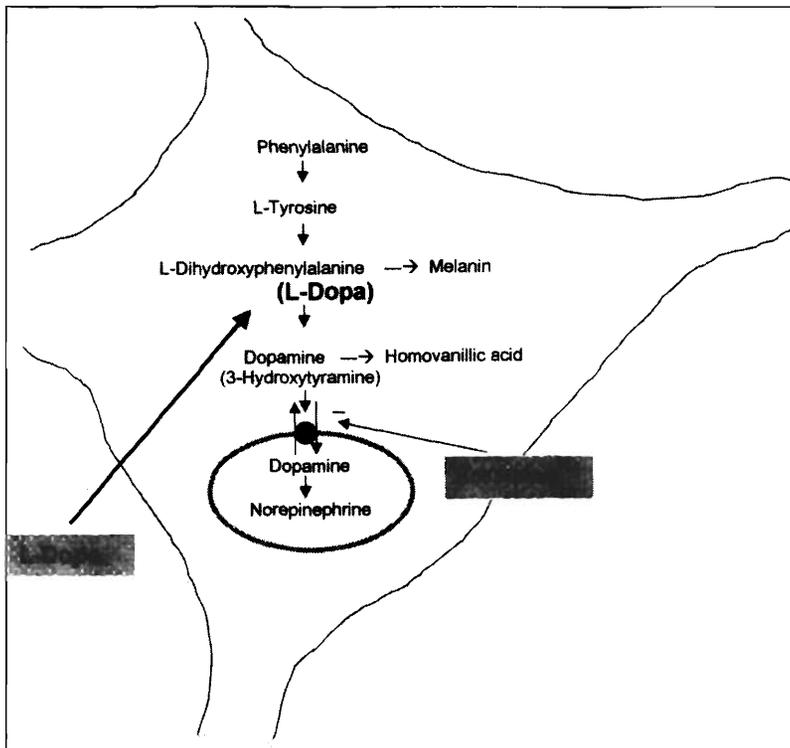
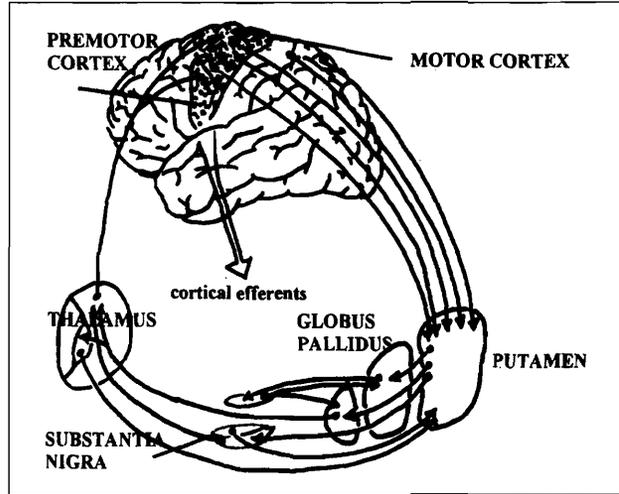


Figure 2. Major pathway for the synthesis of norepinephrine in a neuron. Alleviation of the movement related problems related to reserpine administration by L-dopa, suggested to Dr. Arvid Carlsson that L-dopa by conversion to dopamine could on its own act as a neurotransmitter.

Figure 3. The motor circuit of the basal ganglia i.e., the principal brain regions involved in the control of movement. The dopaminergic connection indicated by arrows from the substantia nigra to the putamen degenerate in Parkinson's disease and manifest as loss of melanin staining in the brain regions. Melanin is a by-product of L-dopa. See Figure 2.



dopamine instead of norepinephrine. This suggested that dopamine, acting as a neurotransmitter caused functional recovery of the animal.

How important was this discovery? We are aware of a neurological disease called Parkinson's disease, which is characterized by rigidity and tremors. Movement control is a complex process requiring interaction and active participation of neurons in the different areas of the brain as indicated in *Figure 3*. Dopamine is found in specific areas of the brain viz. the caudate nucleus, putamen and substantia nigra. The main dopamine carrying neuronal pathway arises in the substantia nigra. Axons from the substantia nigra reach the neo-striatum. Dopamine is stored in synaptic vesicles located in the nerve terminals from which they are released upon stimulation.

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The discovery of dopamine as a neurotransmitter by Carlsson and his group, led in turn to the finding that degeneration of dopamine producing neurons involved in movement control is what causes the tremor, rigidity and akinesia. As a consequence of the initial observations of the reversal of rigidity in reserpine treated experimental animals with L-dopa, L-dopa was developed as a drug against Parkinson's disease and is still used today to treat patients with Parkinson's disease. One of the conse-



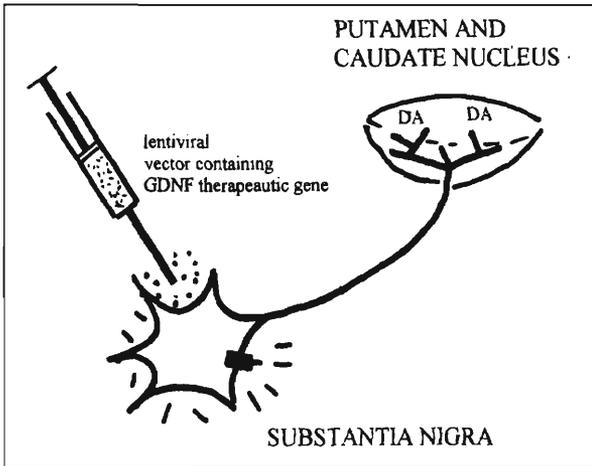


Figure 4. The most recent experiments on monkeys seem to indicate that local application of lentiviral vectors containing the GDNF therapeutic gene, results in sprouting of dopaminergic terminals in the putamen and caudate nucleus of the brain, which are regions affected in Parkinson's disease. The decrease in Parkinsonian symptoms occurred 1-2 months after GDNF gene delivery and lasted for upto 8 months. (Kodower and others, *Science*, 290: 723, 2000)

quences of treatment with L-dopa is the manifestation of psychiatric diseases and this is because the dopaminergic pathways are also involved in regulating behaviour. In Parkinson's disease there is progressive loss of dopaminergic neurons, i.e., neurons that synthesize dopamine and the neuronal loss can be as high as 45% per decade. The cause of neuronal death is believed to occur due to a combination of oxidative stress, mitochondrial dysfunction, excitotoxicity and calcium influx. The long-term solution to the treatment of Parkinson's disease lies in replacing the lost connections. Transplantation of fetal neurons in experimental animal models of Parkinson's disease has been tried and there are reports of human trials as well. The application of gene-therapy in a recent report is schematically shown in *Figure 4*. The use of L-dopa in treatment of Parkinson's disease, subsequently led to the development of antipsychotic and antidepressive drugs, and ushered in an era of a more humane approach to treat patients suffering from these ailments.

How does dopamine act on the neurons? Is it a fast action or a slow action? Fast in terms of neurophysiological function refers to a phenomenon occurring in millisecond range, while a slow response occurs in the range of seconds to hours. The action of the neurotransmitter acetylcholine at the neuromuscular junction on the muscle cell is a fast response and is aptly so, because

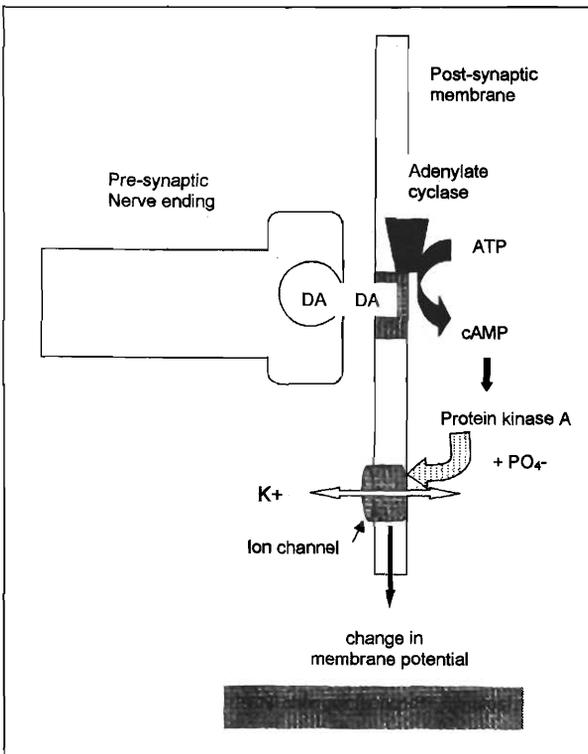
Arvid Carlsson was born on January 25, 1923 in



Uppsala, Sweden, and is currently an Emeritus Professor at the Department of Pharmacology, Goteborg University, Sweden.



Figure 5. Mediation of slow changes in resting membrane potential by dopamine and slow synaptic potential changes following release of dopamine from the presynaptic nerve terminals. Activation of dopamine receptors following dopamine binding results in increased levels of cAMP in the cytosol through enhanced adenylylate cyclase activity. Phosphorylation of ion channels in the membrane, through activation of protein kinase A, results in modulation of ion channel activity and thereby changes in membrane potential.



the contraction of muscles required for retraction of the hand from the source of pain or noxious stimulus such as heat has to be fast. A number of functions of the brain such as mood and alertness however involve slow synaptic transmission. Slow synaptic transmission can also control fast synaptic transmission. Discoveries related to the participation of the neurotransmitter dopamine in slow transmission was essentially made by Paul Greengard. He further went on to show that slow synaptic transmission involves protein phosphorylation. Protein phosphorylation is an important biochemical reaction where coupling of phosphate groups to a protein alters its structure and function. What Paul Greengard showed is that when dopamine acts on the receptor in the postsynaptic membrane it results in the production of the messenger, cAMP inside the cell, which in turn activates a protein called protein kinase A, which adds phosphate groups to other proteins in the nerve cell. An important class of proteins in the membrane of nerve cells is ion

channels. Flow of ions through the channel controls the excitability of nerve cells, which aids in electrical transmission along axons and nerve terminals. *Figure 5* schematically shows the mode of action of dopamine in the post-synaptic membrane to mediate the slow synaptic response. In an exhaustive series of work, Paul Greengard showed the occurrence of phosphorylation in several regions of the brain. Continued research efforts on the phosphorylation mechanisms revealed that dopamine and several other neurotransmitters influence a regulatory protein called DARPP-32 whose role is like a conductor in an orchestra regulating the function of several membrane proteins particularly ion channels, thereby altering their function.

The phosphorylation of proteins by a neurotransmitter, which participates in slow synaptic transmission, has several important functional consequences. One important functional aspect of the brain which has been investigated in molecular details, and whose manifestation has been ascribed to protein phosphorylation, is learning and memory.

Learning can be broadly defined as the process of acquiring knowledge of the world, while memory can be defined as the retention or storage of that knowledge. Cognitive psychologists broadly classify memory into two types, viz. explicit memory and implicit memory. Explicit memory is the memory related to people, places and things, while implicit memory is the memory associated with acquisition of motor/perceptual skills. Implicit memory can be either of the associative type or the non-associative type. Habituation and sensitization, which are responses to repeated exposure to a single type of stimulus, fall under the non-associative memory class. The reader is requested to see an earlier article by Rohini Balakrishnan (see Learning from a Sea Snail: Eric Kandel, *Resonance*, Vol. 6, No. 6, p. 86, 2001).

Eric Kandel used the sea slug *Aplysia californica* to understand the mechanisms of learning and memory. *Aplysia* has comparatively a simpler nervous system with a smaller number of neurons (20,000) compared to billions of neurons in the brain of a mammal such as rat. It has a simple protective reflex that protects its gills, which was used by Kandel to unravel the molecular basis of learning and memory. The premise for using such a simple system was that even simple primitive organisms must learn in order to survive. His earlier experimental system consisted of placing the slug in a chamber containing sea water, and applying controlled jets of water or pressure to the siphon to stimulate it (Figure 6). The extent of the withdrawal of the gill was monitored either by monitoring muscle tension of the gill or by placing a photo-cell beneath the gill, the amount of light reaching the photocell being an indicator of the status of the gill; in a retracted state, more light would reach the photocell. Using this simple system he went on to show the phenomenon of

Paul Greengard, was born December 11, 1925 in New



York, USA and is currently Professor and Head, Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY.

Eric R Kandel was born on November 7, 1929 in



Vienna, Austria is an American citizen. He is currently at the Center for Neurobiology and Behavior, Columbia University, New York, USA.

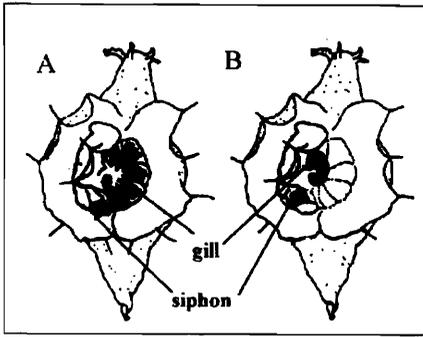


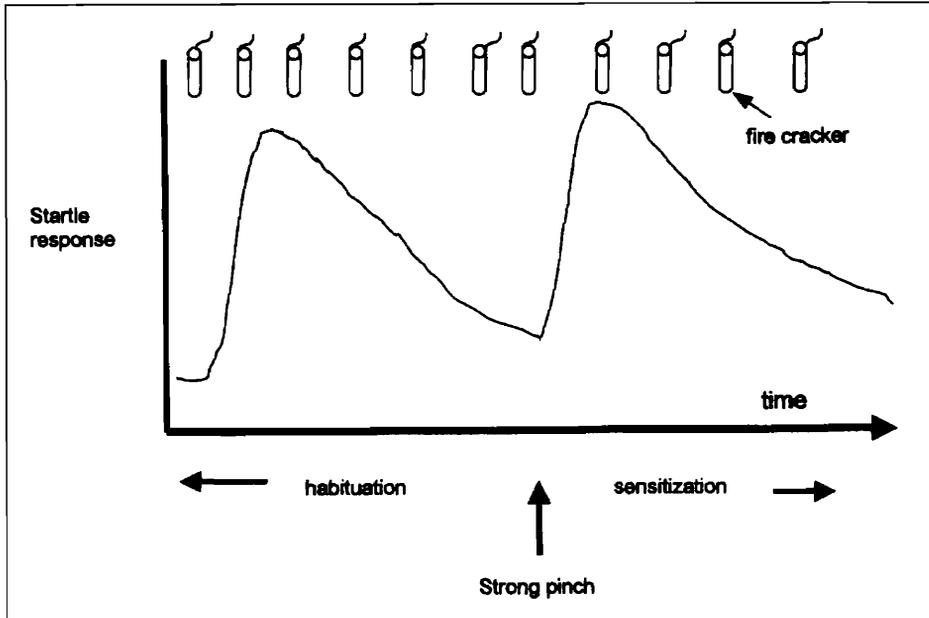
Figure 6. Defensive withdrawal reflex in sea slug, *Aplysia*. Dorsal view of an intact *Aplysia*, with parapodia and mantle shelf retracted to expose the gills and the siphon. Stimulation of the siphon by applying either gentle pressure or a jet of water causes contraction of the gill (B) from the relaxed state shown in A. Kandel essentially used this simple preparation to unravel the molecular basis of learning and memory, by monitoring the electrical activity of neurons in the abdominal ganglion while the animal exhibited the reflex activity.

habituation. Habituation can be explained in a simpler way by considering our response to bursting of crackers on a Diwali evening, the Indian festival of lights. The reaction to the bursting of cracker is plotted as an arbitrary normalized startle response in *Figure 7*. Initially, i.e., the startle response is high, but as the evening progresses the response to cracker bursting diminishes, a phenomenon termed by psychophysicists as habituation. If an adverse stimulus such as pinching is

given during the period of habituation, then the person shows a heightened response to the bursting of a cracker subsequent to pinching, a phenomenon termed as sensitization.

Eric Kandel was able to demonstrate these phenomena experimentally in the *Aplysia*, by applying controlled and constant stimulus to the siphon and monitoring the retraction of the gill. The remarkable achievement of Eric Kandel lays in pushing the question further to understand what neurons participate in the habituation and the sensitization process. Careful experimentation using simultaneous monitoring of gill withdrawal reflex and neuronal activity using intracellular recording techniques which helps probe the electrical changes in a single neuron, helped identify the key neurons and the interneurons located in the abdominal ganglion in the gill withdrawal reflex. Experimentally identifying the participating neurons is indeed a daunting task, it is like trying to figure out how the different chips in a printed circuit board are connected without a circuit diagram at hand. But the problem is comparatively simpler to solve in a simpler system such as *Aplysia* because of the smaller number of neurons involved. An important finding by Eric Kandel was that certain kinds of stimuli resulted in amplification of the protective reflex of the sea slug, and the strengthening of the reflex could remain for days and for weeks and was thus a form of learning. He further went on to show that learning was due to an amplification of the synapse that connects the sensory nerve cells to the motor neurons which activate muscle groups that give rise to the protective reflex. In trying to decipher the phenomenon





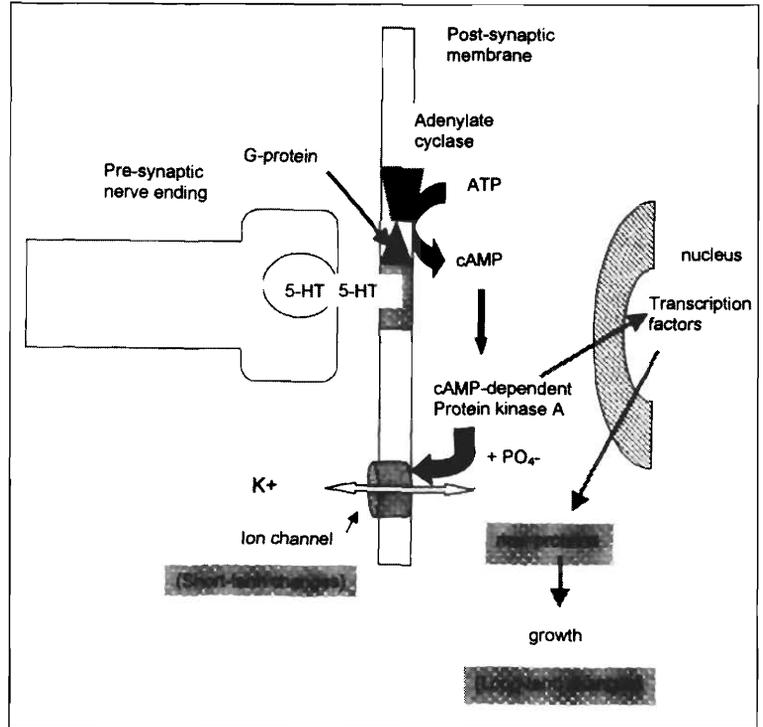
electrophysiologically he used the quantal hypothesis which was used by the Nobel laureate Bernard Katz to understand neurotransmitter release at the neuromuscular junction.

The amplification of the synapse essentially meant that increased amount of neurotransmitter is released at the synapse. It then became important to understand what causes the heightened release of neurotransmitters which are packaged in little sacs, the synaptic vesicles. The advent of the patch-clamp technique, an electrophysiological technique which helps us understand the function of a single bio-molecule, an ion-channel in the membrane of a cell in real time helped further in the understanding of the finer cellular details. It was found that serotonin, a slow neurotransmitter resulted in the closure of a class of ion channels i.e., the K^+ selective ion channels, through phosphorylation by protein kinase A. Activation of protein kinase A occurs by increased levels of cAMP inside the nerve terminal. Closure of K^+ channels manifests electrophysiologically as a broadening of the action potential waveform, which in turn leads to increased influx of calcium through voltage-gated

Figure 7. Habituation and sensitization exemplified by the startle response in a human exposed to the noise of a cracker burst during a Diwali (the Indian festival of lights) evening. During the earlier part of the evening, the response is high and wanes with time, i.e., habituation to the stimulus occurs. A different stimulus e.g. a strong pinch results in a heightened startle response- a phenomenon related to sensitization.



Figure 8. Cellular and molecular mechanisms underlying long-term sensitization in the *Aplysia*, summarizing the findings of Eric Kandel. See text for details.



calcium channels to promote increased fusion of synaptic vesicles with the presynaptic membrane and thereby increase transmitter release. The results are summarized in *Figure 8*.

Subsequently, Eric Kandel's group went on to establish that cAMP can activate transcription factors by reaching the nucleus of the cell and cause changes in several proteins in the synapse, increase in certain proteins and decrease in other proteins. Long-term memory, which we are aware of as human beings, as our ability to recall events that occurred in the distant past, would involve such changes in the levels of proteins in the synapse. He has also demonstrated that the long term changes in synaptic function seen in the sea slug, also occur in the part of the mammalian brain called the hippocampus. Recent studies which include the application of confocal microscopy, indicate that the shape of the synapse can indeed change such that they become larger (*Figure 9*), and are a consequence of the cellular events shown in *Figure 8*. Much of the recent work from Kandel's group

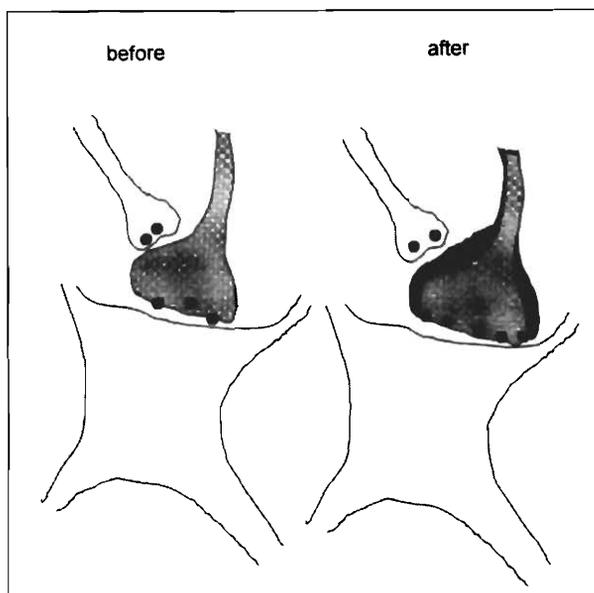


Figure 9. Changes in form and function of the synapse associated with long-term sensitization and memory. This occurs following synthesis of new proteins upon activation of transcription factors in the nucleus of the neuron. The scheme shows a synapse affecting another synapse resulting in increase in size of the nerve ending indicated by dark shading. An important consequence of change in size of the pre-synaptic nerve ending is increase in the efficacy of the synapse resulting in enhanced release of neurotransmitter.

has extended to the use of transgenic mice to understand the precise role of a protein molecule in memory processes. In short, Eric Kandel's work very elegantly demonstrated how nerve cells utilize the slow and very slow synaptic phenomena to store memory.

The work of the three Nobel laureates converges on the central theme of slow signal transduction mechanisms in the neurons. Had there been no identification of dopamine as a neurotransmitter by Arvid Carlsson, it would not have led Paul Greengard to identify its slow mode of action in the super cervical ganglion initially and the accompanying changes in protein phosphorylation. What Eric Kandel went on to show was that, there can indeed be very slow changes in the neurons upon activation by a neurotransmitter in processes such as learning, which utilize the intracellular messenger system worked out by Paul Greengard, with further steps extending to protein synthesis and structural changes. The work of the three Nobel laureates is thus related, and the Nobel Prize well deserved.

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