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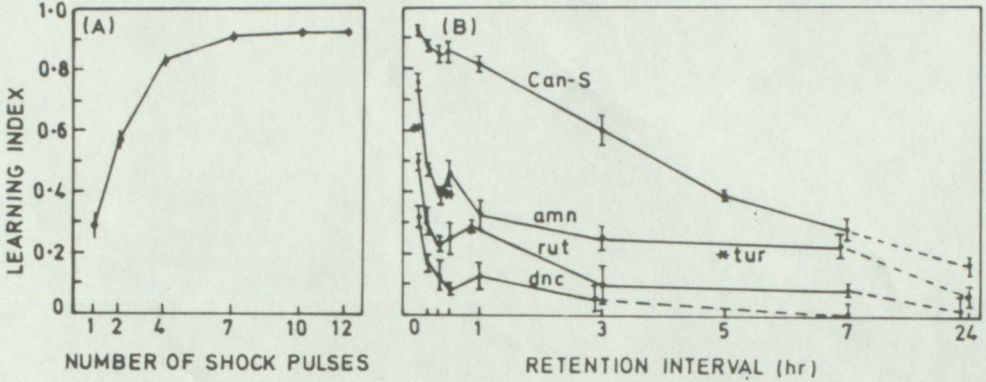

fig 4

I wish to express my gratitude to the Indian National Science Academy for giving me the Aryabhatta award. Aryabhatta, the famous astronomer and mathematician (476 AD) has been described as the creator of Indian Astronomy. To be connected with his name, howsoever indirectly (and undeservingly) at this great distance in time, is indeed a great honour. I take this opportunity to thank the council of INSA and its President for their kindness.

The topic of my lecture, learning and memory is a subject which has interested speculative minds in all ages. Aryabhatta too may have thought about it. Aristotle and St Thomas Aquinas certainly did. It still remains a largely unresolved subject. Some recent developments in molecular neurobiology promise to provide us with insights into biochemical mechanisms of learning. It is to these that I am going to draw your attention.

Learning involves acquisition of new knowledge. Memory has to do with storage of acquired knowledge. Both imply a change in behaviour with experience. This definition is very broad and includes a hierarchy of phenomenon.

Sensitization

Habituation

Associative conditioning (Pavlovian learning)

Higher learning involving cognition and awareness

Is there a common elementary mechanism underlying these very diverse processes? Simplest organisms are capable of elementary forms of learning but, properly speaking, learning requires a certain level of ecomplexity in the system, in particular a set of interconnected neurons. D O Hebb suggested some fifty years ago that in the course of learning, connections between neurons undergo changs. This change in synaptic connections or "synaptic efficacy" is the key to the problem of the memory trace.

Molecular Mechanisms

The first attempts to formulate an explicit molecular theory of learning date to early days of molecular biology. It was suggested that learnt information could be stored in RNA. The claim was that, when trained *Planaria* (flat worms) are mashed and fed to their naive brothers, acquired behaviour is transmitted to cannibals. The vehicle of memory transfer, it was claimed, were the RNA molecules. These irreproducible experiments based on ill-conceived theories soon fell by the wayside.

Molecular Biology of Learning and Memory

O SIDDIQI

National Centre for Biological Sciences, Tata Institute of Fundamental Research

(Delivered on 8 October 1992; Received on, 12 July 1993)

Figure 1 Excitation of nerve cells by external stimuli involves a variety of signalling systems. The diagram summarises recent work on olfactory sensory neurons. A number of second messengers, cAMP, IP_3 and Ca^{2+} can activate kinases which phosphorylate synaptic proteins. Protein modifications accompanying electrical activity of neurons are the basis of short terms memory

Figure 2 Associative memory in neurons arises by strengthening of synaptic connection by (a) coincident activity in the presynaptic and post-synaptic cell (Hebb's hypothesis) and (b) coincident activity of a modulatory neuron synapsing with a presynaptic terminal (Tauc-Kandel model)

Figure 3 Biochemistry of associative conditioning in the gill withdrawal reflex of *Aplysia*. The release of serokonin by a presynaptic modulatory neuron starts a chain of signals leading to protein modifications which increases the efficacy of the synapse between the sensory neuron and the motor neuron

Figure 4 Learning mutants of *Drosophila* Left. The flies can be conditioned to avoid particular odours by means of electric shock Right. Genetic mutants, *dunce*, *nuabaga*, *cabbage* and *amnesiac* are unable to convert short term memory into long term memory. Some of these genes control the pathways of phosphorylation cascade

Red insights into molecular mechanisms of synaptic changes came with the discovery that proteins undergo chemical modification such as phosphorylation of certain amino acids. These modifications are rapid and do not require *de novo* protein synthesis. Activities of protein molecules can thus be changed without recourse to genetic mechanisms. Protein modifications such as phosphorylation or methylation are controlled by small molecules like cyclic AMP, the so called 'second messengers'. Second messengers in cellular signalling provide a plausible mechanism for triggering short term memory in nerve cells.

Certain hormones and neurotransmitters bind to receptors on the cell membrane and activate an enzyme adenylate cyclase which converts ATP to cyclic AMP. Cyclic AMP activates a second set of enzymes, the *kinases* which can phosphorylate proteins. Phosphorylation either increases or decreases the biological activity of target proteins. Cyclic AMP is one of the second messengers. There are others such as IP_3 (inositol 1,4,5, - triphosphate), diacylglycerol or Ca^{2+} which act through other biochemical pathways to connect external signals to persistent internal changes in cellular biochemistry (figure 1).

Phosphorylation of membrane proteins can change the signalling or computational properties of neurons and alter presynaptic and post-synaptic efficacy. There are two major classes of target proteins whose modification affect synapses; cannels which allow the ifnlow and outflow of ions such as Na^+, K^+, Ca^{2+} and Cl^- and receptor-coupled enzymes which are activated by second messengers.

Associative conditioning

Ivan Pavlov, the Russian physiologist, found that when a conditioned stimulus (CS) is paired with an unconditioned stimulus (UCS) repeatedly, the response initially evoked by UCS, can be evoked by CS. For conditioning to occur, CS and UCS must be paired closely in time. Classical Pavlovian conditioning according to Hebb's interpretation requires coincident activity in the presynaptic and the post synaptic neuron. When this coincidence is repeated the synapse is strengthened. In 1963, Kandel and Tauc discovered a new associative rule. They found that connections between two neurons could be strengthened with the help of a third modulatory neuron. The modulatory neuron in the Kandel-Tauc model acts on the presynaptic terminal. Coincident activity in the presynaptic neuron and the modulatory neuron changes the strength of the synapse. The two situations are diagrammed in figure 2.

Gill Withdrawal Reflex in *Aplysia*

The molecular mechanism of synaptic strengthening was first worked out by Kandel and his associates, working with the sea hare *Aplysia californica*. *Aplysia* has about 20,000 large neurons which can be recognized individually and impaled with electrodes. This permits a detailed analysis of the neural circuits which underline various aspects of *Aplysia's* behaviour. One of these behaviours, much studied by physiologists, is the gill-withdrawal reflex stimulated by touching the siphon or the mantle shelf. The response (CS) can be strongly conditioned by pairing it with an electric shock to the tail (UCS). About five trials are enough to induce learning. The behavioural circuit is outlined in figure 3. A modulatory neuron releases serotin which acts on the presynaptic terminal of the sensory neuron. Serotorin released by the UCS activates adenylate cyclase in the presynaptic terminal of the sensory neuron, cyclic AMP in turn activates a protein kinase which phosphorylates K^+-channels to cause prolonged depolarization and calcium influx. Incoming calcium binds to calcium calmodulin and further activates adenylate cyclase—a kind of positive feed back. Through a second pathway involving phospholipase and protein kinase C, synaptic vesicles are emobilized to release neurotransmitter at the motor neuron. This, somewhat complicated biochemistry leads to increased synaptic efficacy. A second stimulus to the sensory neurons of the siphon elicits a stronger motor response thanks to protein phosphorylations caused by previous experience.

The fruitfly, *Drosophila melanogastor* can be trained to avoid specific odours by punishment with electric shock. W G Quinn isolated a number of mutants with impaired learning and memory. These mutations have been shown to affect various steps in cAMP-dependent phosphorylation. In the mutant called *dunced* the enzyme phosphodiesterase is deffective. The dunce flies are unable to consolidate long term memory. In the mutant *rutabaga* a calcium-calmodulin dependent adenyl cyclase is affected. It has been shown recently that the enzymes of phosphorylation metabolism involved in the learning pathway are concentrated in the mushroom body, a part of the fly's brain which is believed to be the association centre. Analysis of learning mutants of *Droso-phila* thus leads us to the same biochemical mechanisms that are revealed by neurophysiological experiments on the gill withdrawal reflex of *Aplysia* (figure 4).

Hebbian Synapse and NMDA–Receptors

Synaptic strengthening of the type postulated by D O Hebb has been demonstrated in the hippocampus of vertebrates. Hippocampus is a temporary repository of visual memory in its passage from sensory to pre-frontal cortex. Neurons of hippocampus display plastic behaviour characteristic of learning. Stimulated electrical activity in the hippocampal pathways leads to increased synaptic efficacy. The phenomenon is called long term potentiation (LTP), it can be studied *in vitro* in slices of hippocampus and is currently the object of intensive world wide research. Associativity in the hippocampal pathways is of the classical Hebbian type, a presynaptic to post-synaptic strengthening. Its molecular basis is somewhat different from that of Pavlovian conditioning. LTP is mediated by glutamate and N-methyl-D-aspartate (NMDA) receptors. The NMDA channel is blocked in the resting state by Mg^{++}. If the neuron is depolarised, let us say, by the activation of some non-NMDA channel, the NMDA channel is unblocked leading to an influx of calcium and the activation of the second messenger system. NMDA channels thus act as coincidence detectors with properties analogous to adenylate cyclase. There is an important difference. Induction of LTP depends upon post-synaptic calcium influx but the maintenance of LTP requires an enhanced presynaptic release of transmitter. A signal must, therefore, go from the post synaptic to the presynaptic terminal. The messenger for this retrograde signal is believed to be nitric oxide.

Higher Learning

In 1950, Brenda Milner described a remarkable case of amnesia in a patient after bilateral removal of temporal lobes. The patent lost the ability to form new long term memories although he retained previously acquired memories. This kind of memory loss has been extensively investigated in men and monkeys by Mishkin and others. These studies throw light on the role of temporal lobe in memory formation.

Visual memory formation in primates starts from the striate cortex, where a topographic map of the visual field is laid down. This is the so called 'primal sketch' of David Marr. A more global representation of the visual scene is then transmitted to the inferior temporal cortex whose role has been greatly clarified by experiments involving temporal lesions. There seem to be two parallel pathways running through hippocampus, amygdala and dienceptalon. Bilateral lesions of hippocampus and amygdala cause global amnesia. If hippocampus alone is damaged 'spatial memory' is affected but 'object memory' remains intact. Amygdala on the other hand is critically necessary for associating visual memory with memories in other modalities. We have not even begun to understand the detailed mechanisms of global memory but proteins whose synthesis and modifications are correlated with memory formation in the brain of higher animals are at present, the object of active research. One such protein is called F1. It has been shown that phosphorylation of F1 by protein kinase C follows the pathway of visual memory in the temporal lobes.

Conclusion

Short term changes in synaptic efficacy are accompanied with molecular modifications in proteins. Phosphorylation has a pronounced effect on the activity of synaptic proteins. Different forms of learning and memory, associative learning, habit formation or cognitive learning may employ somewhat different pathways but the elementary biochemical mechanisms are probably universal.

Maintenance of memories over long durations requires other, stable alterations in the wiring diagram of the brain, number of functional receptors, number and location of synapses or size and extent of neuronal arborizations. The molecular mechanisms that I have discussed have the attractive feature that they allow us to connect short term changes with long term changes in a natural way through gene activation. At any rate we seem to have come a long way from the neuropsychology of yesteryears which looked upon cortex as an ill-defined tissue and the memory trace as an enigma.