REVIEW AND COMMENTARIES

Molecular mechanisms of memory and learning

Dorothea Kapoukranidou, Harilaos Papachristou, Epameinondas Gousopoulos

Laboratory of Physiology, Medical Faculty, Aristotle University of Thessaloniki

ABSTRACT: The aim of the present review is to discuss the molecular mechanisms of learning and memory. The first part of the review investigates implicit memory in Aplysia, a marine snail, studied by Eric Kandel, the Nobel Prize winner. This form of learning can be broadly divided into two temporal phases, an early and a late phase. The molecular mechanisms of each phase will be analyzed in separate sections.
The second part of the review investigates hippocampal-dependent explicit memory in mammals and the mechanism that underlies it, known as long-term potentiation (LTP). Similar to the molecular mechanisms in Aplysia, LTP is divided into an early and a late phase. However, LTP in mammals is a very complicated phenomenon that depends on the regulation of many molecular pathways. Moreover, the scientific community cannot always reach a consensus on the role of some of these molecular mechanisms in LTP. Nevertheless, it will be demonstrated that both explicit and implicit memory occur at a synaptic level, result from/in synaptic strengthening, and share common molecular mechanisms in a variety of species far apart on the phylogenetic scale.

Key Words: Memory, Learning, Molecular mechanism, NMDA receptors.

In the field of psychology and of behavioural sciences, the term *learning* refers to the process of acquiring knowledge and skills, while the term *memory* represents the organism's ability to store, maintain, and retrieve this knowledge[1,2].

With reference to humans and on the basis of what is learned and how it is learned, we can classify memory into *declarative* or *explicit* and *non-declarative* or *implicit* memory[3]. Declarative memory requires conscious effort and awareness. It refers to the conscious recall of facts, faces, places, objects, and events. In contrast to declarative/explicit memory, non-declarative or implicit memory is automatic, without awareness, and is usually expressed through performance. In general, it includes reflexive learning such as habituation, sensitization, and classical conditioning as well as learning of motor skills such as when we tie our shoelaces or ride a bicycle[4,3].

A different criterion for the classification of memory is its temporal duration. Thus, a very broad temporal classification of memory is into *short-term memory* (STM) that lasts from few seconds to minutes, and into *long-term memory* (LTM), whereby the short-term mnemonic information becomes consolidated and stored in a durable form with repetition/rehearsal[1,3].

At a neural level, learning changes the function and the structure of the central nervous system (CNS). These neural changes represent our memories[5]. Investigations in invertebrates and mammals examined different memory systems but they have come to similar conclusions.

As regards to invertebrates, Eric Kandel, the Nobel Prize winner, experimented with the sea snail *Aplysia Californica*[4]. He and his many associates focused on reflexive learning such as habituation, sensitization, and classical conditioning, which as mentioned above, are types of implicit memory[4,6,7].

As regards to mammals, research in rodents focused on long-term potentiation (LTP) and the hippocampal system, a neural structure deep in the temporal lobes. LTP and the hippocampus play a key role in explicit spatial memory in rodents[5,6]. Both lines of research have shown that learning and memory occur at a synaptic level and result in/from synaptic strengthening and growth[7].

Corresponding author: Dorothea Kapoukranidou, Tel.: +30 2310 999328, +30 2310 999239, e-mail: dkapoukr@auth.gr

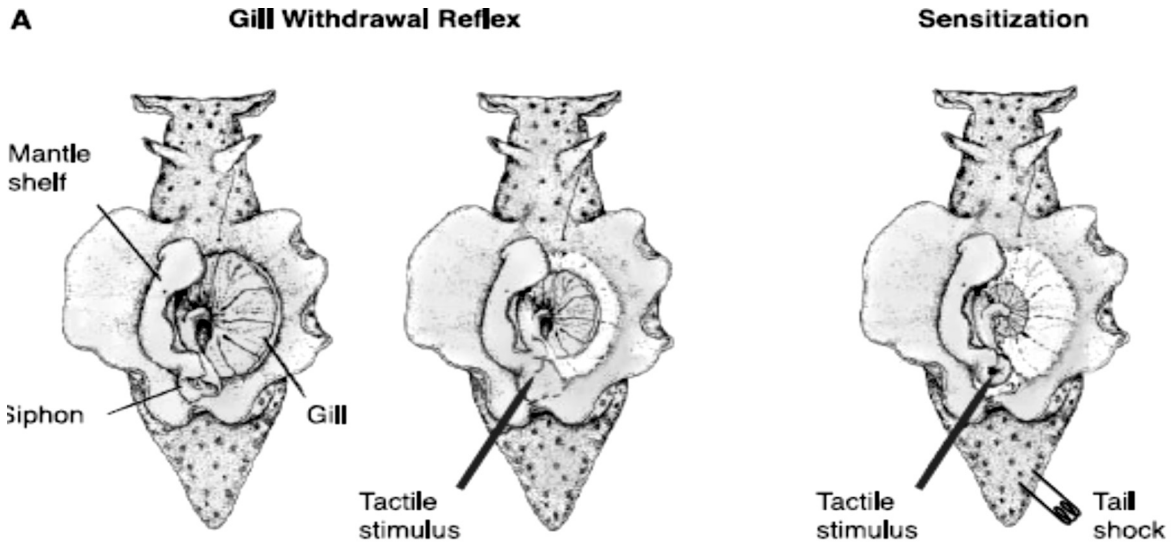

Figure 1. Schematic representation of the gill-withdrawal reflex and sensitisation in Aplysia (Kandel, 2001, Science).

Short-term Implicit memory in Aplysia

Kandel made his discovery by studying Aplysia, a marine snail. What makes it attractive is that its CNS is remarkably simple. It consists of about 20.000 nerve cells. Moreover, its cells are large enough to be seen with the naked eye and easy to stimulate and monitor with electrodes in the laboratory[7].

His aim was to simulate in Aplysia the forms of reflexive learning that Pavlov had studied in his animal experiments, namely habituation, sensitization, and classical conditioning[4,6]. Kandel and his associates experimented with the simplest behaviour in Aplysia; the gill-withdrawal reflex[4,6].

The gill is the organ that Aplysia uses for respiration. It is located in the mantle cavity, and is protected by the mantle shelf. The mantle shelf ends to a fleshy protrusion, called the siphon, which enables the mantle cavity to get rid of waste and seawater[4,7]. A light touch either to the siphon or to the mantle shelf results in the gill-withdrawal reflex, a defensive withdrawal of the gill into the mantle cavity for protection from possible injury[4,6,7] (Figure 1). However, with repeated touching the reflex becomes gradually weaker as the animal learns to perceive the stimulus as unimportant. At this moment, habituation to the tactile stimulus has occurred[6].

Sensitization is a type of learned fear and develops when a strong shock is applied to Aplysia's tail[7]. The noxious stimulus (shock) triggers an exaggerated gill-withdrawal reflex. If after a short time a harmless tactile stimulus is applied to the siphon, it will elicit the same exaggerated response triggered earlier by the strong shock. This is evidence that sensitization has been established[4,6,7](Figure 1).

Finally, the classical conditioning protocol involves the association of a noxious stimulus to the tail with a light touch to the mantle shelf so that the weak stimulus (light touch) comes always first and the strong stimulus (shock) always follows. As a result of this contingency, the light touch operates as a warning for the shock. The association of these two events leads to a stronger gill-withdrawal reflex than in sensitization[4,6,7.]

As far as the neurobiology of the gill-withdrawal reflex is concerned, the sensory neurons that innervate the siphon project directly to the motor neurons that activate the gill[4,6,7]. However, the sensory neurons also form indirect connections with the motor neurons through interneurons (Figure 2). A light touch to the siphon activates the sensory neurons, which release glutamate and generate excitatory postsynaptic potentials (EPSPs) to the motor neurons and the interneurons. The motor neurons in turn trigger the gill-withdrawal reflex. However, after a brief training session of 10 presentations of the neutral tactile stimulus, the EPSPs on the motor neurons become progressively weaker

and this decrease in the intensity of the gill-withdrawal reflex can last for minutes. This is the result of a decrease in the amount of glutamate released by the presynaptic terminals. Therefore, short-term habituation reveals a reduced synaptic transmission between sensory and motor neurons as well as interneurons[7].

Similarly to short-term habituation, short-term sensitization takes place after only one training session and can last for several minutes. Unlike habituation, sensitization results from an increase in glutamate release from the sensory neurons[6,7]. However, the neural circuit involved in sensitization is more complicated than in habituation. The sensory neurons that innervate the tail synapse on modulatory serotonergic interneurons. Moreover, the modulatory interneurons form synapses not only with the cell bodies of the sensory neurons of the siphon but also with their terminal buttons (axoaxonic synapses)[6,7] (Figure 2).

The modulatory interneurons stimulate the sensory neurons of the siphon to release glutamate. They do so by increasing the amount of the second messenger cyclic adenosine monophosphate (cAMP) in the terminal buttons of the sensory neurons[6]. The whole process can be described as follows: First, serotonin (5-HT) binds to serotonergic metabotropic receptors and activate a G protein (Gs) located in the interior of the cell membrane. Following this, the active Gs components stimulate the enzyme adenylate cyclase (AC), which transforms ATP into cAMP[8]. AC continues making cAMP for several minutes, thus, elevating cAMP levels in the presynaptic terminal even after 5-HT action has been terminated[4,7]. Furthermore, cAMP turns on the cyclic AMP-dependent protein kinase, or protein kinase A (PKA). The PKA molecule is composed of 4 subunits, two regulatory and two catalytic. The purpose of the catalytic units is the phosphorylation of target proteins[4].

Phosphorylation is the relocation of a phosphate group (PO_4^{-2}) from ATP to one or more hydroxyl groups (OH^-) of serine, threonine, or tyrosine residues in the amino acid chain of a protein[8]. By changing the conformation of the protein molecule, phosphorylation increases or decreases its biological activity[8].

On the other hand, the regulatory components of the PKA molecule normally inhibit its catalytic components. Additionally, the regulatory units contain

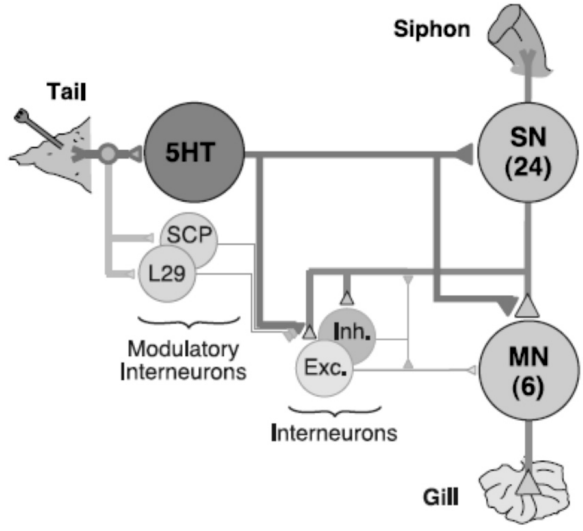

Figure 2. Neurobiology of the gill-withdrawal reflex in Aplysia (Kandel, 2001, Science).

cAMP binding sites. When cAMP levels in a cell become elevated, cAMP molecules bind the PKA regulatory units, change their conformation, and free the catalytic units, which are ready to phosphorylate target proteins[4].

One of the targets of cAMP and PKA is potassium (K^+) channels in the cell membrane. The phosphorylation of the K^+ channels slows down their opening and delays the repolarisation of the cell membrane after an action potential has occurred. The increase in the duration of the action potential permits more Ca^{2+} to enter the cell through N-type Ca^{2+} channels[6,7]. Consequently, the elevated levels of Ca^{2+} enhance glutamate release from the presynaptic terminals. Additionally, cAMP and PKA act through a Ca-independent pathway that facilitates the movement of the glutamate vesicles to the active zone and enhances the efficiency of the exocytotic release machinery in the presynaptic terminals[6]. Moreover, PKA phosphorylates L-type Ca^{2+} channels. The flow of Ca^{2+} ions through this channel also mobilizes the synaptic vesicles towards the active zone but this time through a Ca-dependent mechanism[6]. Finally, there is evidence that the last two biochemical mechanisms are accomplished through the synergistic action of the PKA and another protein kinase, the PKC[6,7]. PKC is also activated by 5-HT through a different type of a serotonergic metabotropic receptor.

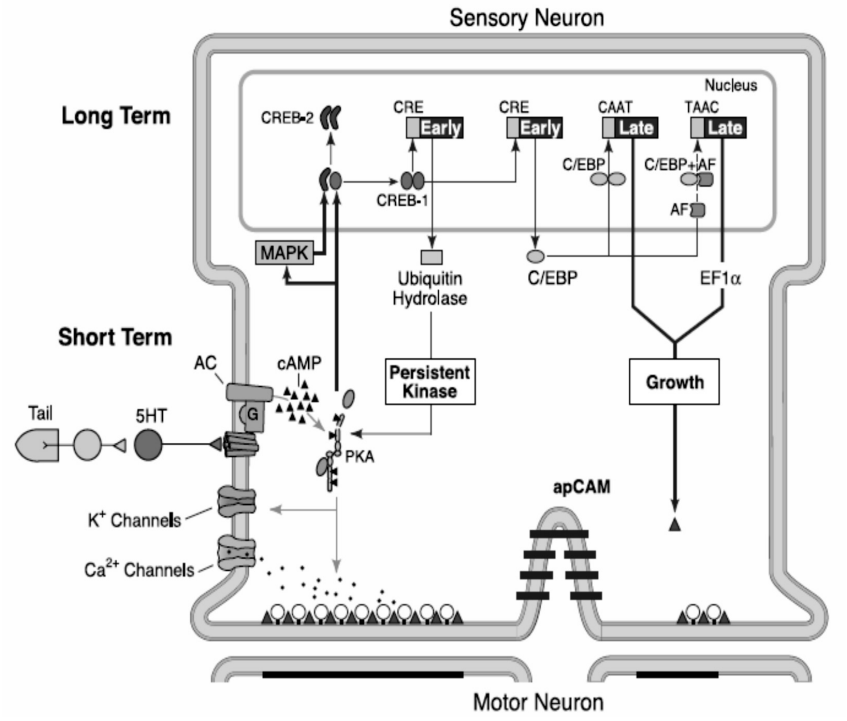

Figure 3. Molecular mechanisms in short-term and long-term memory in Aplysia (Kandel, 2001, Science).

In this receptor, 5-HT activates a different type of G protein (Go), which in turn activates phospholipase C. Phospholipase C causes the release of the second messenger diacylglycerol (DAG). Finally, DAG activates protein kinase C (PKC)[6,7,8].

The neurobiological circuit involved in classical conditioning is even more complicated than in sensitization. The protocol consists of a contingency between two events, a shock to the tail, which is the unconditional stimulus (US), and a light touch to the mantle shelf, which is the conditional stimulus (CS). As mentioned above, the CS always precedes the US. In the control group, the animal's siphon is lightly stimulated but this stimulation is not associated with any other event[4]. The US activates both the sensory neurons that innervate the tail and the modulatory interneurons that form axoaxonic synapses with the sensory neurons of the mantle shelf and the siphon[6,7].

The key molecular component here is the entry of Ca^{2+} in the presynaptic terminals of the sensory neurons that are stimulated by the CS[6,7]. Ca^{2+} binds and activates an intracellular Ca^{2+}-binding protein known as calmodulin (CaM). The Ca^{2+}/CaM complex activates a variety of enzymes such as the AC. By altering the molecular conformation of AC, the Ca^{2+}/CaM complex increases its biological activity and makes it capable of synthesizing more cAMP[6]. Moreover, the US stimulates even further the production of AC through the 5-HT mechanism described earlier in sensitization. Therefore, AC responds both to the CS (binding to the Ca^{2+}/CaM complex) and the US (Gs activates AC after 5-HT binding to its receptor). Greater amounts of cAMP activate greater amounts of PKA which in turn lead to greater amounts of glutamate and thus increased synaptic function[6,7] (Figure 3).

Long-term implicit memory in Aplysia

As mentioned above, a brief training session results in short-term habituation or sensitization. However, 4 such sessions dispersed in time result in long-term habituation and sensitization lasting for several weeks[4]. Furthermore, short-term sensitization in Aplysia requires functional synaptic changes in the form of increased glutamate release from the presynaptic terminals. However, long-term sensitization requires both functional and structural changes at the synapse[7,9].

Structural changes, such as the growth of new synapses, demand protein synthesis and gene expression. In long-term sensitization, repeated applications of the aversive stimulus cause more 5-HT to be released from the presynaptic terminals of the modulatory interneurons. Consequently, more cAMP is produced and more PKA is activated[4]. Furthermore, PKA recruits another protein kinase, the MAP kinase (MAPK)[9]. Following this, PKA and MAPK translocate to the cell nucleus where they phosphorylate a regulatory protein called cAMP-response element-binding protein (CREB)[9]. CREB is a transcription factor (TF), that is, a protein that when phosphorylated binds to the promoter region of a gene and either activates or inhibits its expression. CREB is the TF that mediates cAMP transcriptional activation and binds to a regulatory site called cAMP response element (CRE)[8]. However, there are two types of CREB, CREB-1 and CREB-2. The former stimulates gene expression, whereas the latter suppresses it. In long-term sensitization PKA phosphorylates CREB-1, while MAP kinase phosphorylates CREB-2[4,9].

The regulation of genes by CREB-1 causes both functional and structural synaptic changes. As regards functional changes, CREB activates a gene that encodes an ubiquitin hydrolase necessary for the proteolysis of the regulatory subcomponents of the PKA. Inactivation of the inhibitory subcomponents leads to constant PKA activity[7]. PKA, in turn, can continue the phosphorylation of its substrates, including also CREB-1, even after cAMP levels have been decreased[4,7].

As regards structural changes, CREB-1 activates a second gene that produces a second TF, named C/EBP, which binds to the response element CAAT and activates genes responsible for synaptic growth[7]. Although these CREB-1 effects result in central transcription and translation, evidence also indicates a key role for local protein synthesis in long-term synaptic growth[4,9]. Inhibition of local protein synthesis at the synapse enabled the initial growing of new synaptic terminals but the growing could not be sustained in the long-term[4,9]. Therefore, there must be two mechanisms, one responsible for the initiation of the synaptic growth, and a second one responsible for its maintenance. It seems that the former requires central transcription and translation initiated by CREB-1, whereas the latter depends on local protein synthesis[9]. However, the mRNA molecules needed for local protein synthesis are sent to all the cell's terminals, whereas the structural changes that accompany long-term memory are confined to specific synapses. Therefore, there must be a molecular signal that tags the synapses where local protein synthesis takes place or in other words the synapses affected by learning[4,9]. The process involves biologically inactive mRNA molecules produced in the nucleus and sent to all the cell's terminals. Being in an inactive form they cannot activate local protein synthesis at the synapse [9]. In order to do that, they have to be activated by an homolog of the prion-like protein called cytoplasmic polyadenylation element-binding protein (CPEB) found in the CNS[9,10,11]. However, the genes that encode CPEB produce a recessive form of the protein and not a dominant one. Nevertheless, the increased levels of 5-HT during long-term sensitization transform the dormant CPEB into a dominant self-maintained form that no longer needs continued activation from 5-HT [9,11]. Furthermore, the dominant CPEB transforms recessive CPEB into a dominant form. Finally, the dominant CPEB activates the dormant form of mRNA that gives rise to new protein synthesis at the specific synapse and leads to synaptic growth[4,9,10,11] (Figure 3).

Long-term potentiation and explicit memory in mammals

Many types of learning are associative in nature. In daily life, an organism learns to associate a variety of stimuli with each other and with different responses and reinforcers. More than fifty years ago, the Canadian psychologist Donald Hebb proposed a theoretical rule that expresses this idea at a molecular level[5, 8]. The *Hebb rule* states that if a synapse is repeatedly activated at about the same time that the postsynaptic neuron is active, structural and chemical changes will occur at the synapse that will make it stronger[5,8]. Research on the phenomenon of long-term potentiation (LTP) in hippocampus has confirmed Hebb's rule validity[1].

The hippocampal formation is a part of the limbic system that includes the sectors of Ammon's horn (cornu Ammonis or CA), the dentate gyrus (DG), and the subiculum. The entorhinal cortex (EC) channels

the major neocortical inputs through the perforant path to the granule cells of the DG. The DG, in turn, relays the incoming information to the pyramidal cells of the field CA3 of the Ammon's horn (mossy fiber projection). Moreover, neurons in the field CA3 project to the field CA1 of the hippocampus (Shaffer collaterals) and to other forebrain structures such as the septum and the mammilary bodies[12].

In 1973, Bliss and Lomo discovered that a brief, high-frequency electrical stimulation of the axons in the perforant path increases the size of the EPSPs in the DG; this increased synaptic response represents a strengthening of the synapse that remains that way from few hours to several weeks and is called LTP[12]. However, LTP can be induced only when the axons are stimulated at a high frequency (approximately 100-400Hz, called tetanic stimulation)[8].

The crucial link between LTP and the Hebbian rule is the existence of an associative form of LTP[12]. When weak and strong synapses on a single neuron are simultaneously activated, the weak synapse becomes stronger. This event is called associative or hebbian LTP because it results from the simultaneous activation of the two sets of synapses[2,5,8].

The establishment of associative LTP requires two events to occur simultaneously, i) the two synapses should fire at about the same time and ii) the postsynaptic membrane should be depolarized. It has been found that LTP in the field CA1 and the DG is associative in nature and results from the special properties of the postsynaptic N-methyl-D-aspartate (NMDA) glutamate receptor[7,5].

The NMDA glutamatergic receptor complex contains a calcium channel which is normally blocked by magnesium ions (Mg^{2+}). Mg^{2+} prevents Ca^{2+} entry even after glutamate binding to the NMDA receptor complex has occurred. However, if the postsynaptic membrane becomes depolarized, then Mg^{2+} is ejected and Ca^{2+} enters the cell. It seems that the NMDA receptor is the molecular mechanism that underlies the Hebb rule; it detects the association of a presynaptic (glutamate release) with a postsynaptic event (depolarization of the membrane)[13].

If LTP mediates hippocampus-dependent learning, then disruption of NMDA-mediated associative LTP will lead to disruption of explicit learning. In support of this hypothesis, the NMDA antagonist 2-amino-5-phosphonopentanoic acid (AP5) blocks LTP in the field CA1 and the DG and disrupts hippocampus-dependent learning in rodents[5,8]. Furthermore, mutant mice lacking the NR1 gene failed to develop NMDA receptors in the field CA1 of the hippocampus. These knockouts were deficient in both hippocampus-dependent learning (spatial learning) and in LTP compared to wild-type animals[13,14,15].

Although the above findings strongly support a key role of NMDA receptors in associative LTP and hippocampal learning, a study by Bannerman and coworkers casts doubt on this relationship. In this study, AP5 blocked NMDA-mediated hippocampal LTP in rats but the animals were still able to learn the Morris water maze (a spatial learning task), provided that they were pretrained in a similar water maze task. These results imply that spatial learning consists of many dissociable pharmacological components and perhaps only some of them depend on NMDA-mediated LTP[16].

Similar to Aplysia, LTP is broadly divided into two temporal phases; an early phase (E-LTP) lasting approximately 1-3 hours, and a late phase (L-LTP) lasting at least one day where protein synthesis and RNA translation are necessary[4]. A brief tetanic stimulation results in E-LTP, whereas four such sessions at 10-minute intervals elicit L-LTP[4]. In the following sections, we will try to elucidate some of the molecular mechanisms underlying these two forms of LTP and to examine their role in hippocampal-dependent learning. However, we should bear in mind that the molecular cascades involved in LTP are not clear-cut and there is no consensus on their role in E-LTP and L-LTP.

Ca^{2+} and CaM-KII

An important step for the establishment of LTP is the entry of Ca^{2+} into the postsynaptic neuron. Ca^{2+} influx is essential for both postsynaptic and presynaptic molecular events that occur during E-LTP and L-LTP[4,13,5].

As regards postsynaptic changes, there is an increase in the number and sensitivity of α-amino-3-hydroxy-5-methyl-isoxazoleproprionic acid (AMPA) receptors in the field CA1 and the DG[5]. Liao and coworkers demonstrated that before the establishment

of LTP in the field CA1, some dendritic spines were populated exclusively with NMDA receptors. These synapses, called silent synapses, lack AMPA receptors at resting potential. However, after LTP induction, the same dendritic spines became populated with AMPA receptors[17].

The trigger for these changes is the binding of Ca^{2+} to CaM. Ca^{2+}/CaM complex, in turn, activates type II calcium calmodulin-dependent protein kinase (CaM-KII)[4].

CaM-KII is derived from four subunit genes (α, β, γ, δ). The α and β isoforms are commonly found in the brain[14]. After activation, CaM-KII is capable of autophosphorylation by attaching a PO_4^{-2} group to the threonine residue at the 286/287th positions of the α and β isoforms respectively (Thr286/287). Once autophosphorylation has been achieved, CaM-KII remains active even after Ca^{2+} has returned to basal levels[14].

CaM-KII and the process of autophosphorylation are important early components of LTP[13,15,24]. CaM-KII inhibitors block E-LTP in the field CA1 of the hippocampus[13,24]. Furthermore, mutant mice lacking the CaM-KIIα isoform showed both a decreased magnitude of E-LTP in the CA1 area and an impaired performance in the Morris water maze task[18,19]. Finally, a complete elimination of LTP was achieved when autophosphorylation was blocked by substituting threonine with alanine at the 286th position in the CaM-KIIα isoform[20].

Once phosphorylated, CaM-KII translocates to the postsynaptic density and binds to the existing AMPA receptors there. The AMPA GluR1 subunit contains a phosphorylation site, Ser831, that when phosphorylated by CaM-KII increases AMPA channel conductance and renders the receptor more sensitive to glutamate[14,21]. Moreover, CaM-KII facilitates the delivery of additional AMPA receptors in the silent synapses. This regulation may occur at two levels; the trafficking process that regulates the entry and removal of receptors from the neuronal membrane, and the anchoring process that creates new unfilled anchoring sites in the plasma membrane and keeps receptors in the synapse[14,21,22]. The increase in the number of AMPA receptors strengthens the synapse.

As regards presynaptic changes, CaM-KII phosphorylates synapsin I in the presynaptic terminals. Phosphorylation of synapsin I dissociates synaptic vesicles from this enzyme and mobilizes them towards the active zone. This effect, in turn, facilitates exocytosis and neurotransmitter release during LTP[13,23].

Finally, CaM-KII appears to play a role in synaptic tagging and local protein synthesis. As described earlier, local protein synthesis is important for structural changes in the late phase of sensitization in Aplysia and CPEB is the local molecular signal that mediates these changes. In rodents, α-CaM-KII mRNA is found in dendrites and its concentration increases during LTP. Most important, CaM-KII has been found to phosphorylate and activate CPEB in dendrites. Therefore, CaM-KII may be one molecular mechanism involved in L-LTP and local protein synthesis in the mammalian hippocampus. Similar to Aplysia, this process may be mediated via CPEB phosphorylation[4,23].

Dopamine, AC and cAMP

As mentioned earlier, the hallmark of L-LTP is protein synthesis and RNA translation. For example, blocking protein synthesis with protein inhibitors interferes with the establishment of L-LTP but not of E-LTP[13,24].

Kandel argues that the molecular cascade leading to protein synthesis is similar to that in invertebrates like Aplysia. That is, the AC/cAMP-mediated pathway is responsible for protein synthesis and LTM in the mammalian brain[4,13].

In support of this hypothesis, Huang and Kandel[25] found evidence for a role of dopamine D1/D5 receptors in hippocampal L-LTP. Dopamine (DA) agonists can simulate L-LTP in the CA1 area, while the specific D1/D5 antagonist SCH23390 blocks it. Moreover, it is known that the dopaminergic system innervates the CA1 field and that DA activates the AC/cAMP pathway. As a result of the above, the authors argued that their findings support the role of AC/cAMP cascade in L-LTP in the CA1 field[24,25].

Consistent with these findings is the role of adenylyl cyclases (ACs) in L-LTP. Hippocampal ACs can be activated by either CaM or stimulation of D1/D5 receptors[24]. Pharmacological studies showed that the AC activator forskolin triggers L-LTP in the perforant path[26]. Additionally, knockout mice deficient in both AC1 and AC8 exhibit no L-LTP or LTM. Moreover, overexpression of AC1 in mutant mice potentiated L-LTP and memory. Furthermore, L-LTP was more

easily established in the mutants than in wild-type animals[23].

PKA

After LTP induction, there is an increase in cAMP and PKA levels and the time course of changes in PKA levels positively correlates with changes in cAMP concentration[13,24].

PKA plays a role in E-LTP by phosphorylating inhibitor 1 (I-1). Activation of I-1 inhibits the phosphorylation of protein phosphatase 1 (PP1). PP1 normally catalyzes the dephosphorylation of AMPA receptors at Ser845, which is also a PKA phosphorylation site. Deletion of both CaM-KIIα and PKA phosphorylation sites in GluR1 subunits caused deficits in both spatial memory and hippocampal synaptic plasticity in mice[23].

The latter finding emphasizes the importance of convergence points in LTP. In this case, two independent pathways, CaM-KII and PKA converge on the phosphorylation of a common effector, the AMPA receptor[23]. The integration of different molecular signals is also emphasized by the fact that PP1 dephosphorylates CaM-KII at Thr286. Therefore, phosphorylation of I-1 by PKA may also lead to longer-lasting CaM-KII effects[27].

On the other hand, L-LTP, but not E-LTP, was profoundly impaired in R(AB) transgenic mice that exhibited deficits in both PKA activity and long-term memory and learning[28]. Furthermore, it was shown that the PKA inhibitor Rp-cAMPS blocks L-LTP in the perforant path[26]. It is assumed that PKA exerts its effect on L-LTP by directly phosphorylating CREB at Ser133[13,24,29]. In agreement with this finding, application of forskolin in the CA1 field was accompanied by PKA activation and increased CREB phosphorylation. However, the MAPK kinase (MEK) inhibitor U0126 blocked the forskolin-induced CREB phosphorylation[29]. This finding implies that the MAPK/ERK cascade mediates LTP and CREB phosphorylation and perhaps PKA action[23,29].

MAPK/ERK as a convergence point

Evidence for the role of MAPK/ERK in LTP comes from pharmacological studies. The MEK inhibitors, PD098059, U0126, and SL327, inhibit L-LTP in the field CA1 of the hippocampus[29]. Moreover, induction of LTP in the DG and CA1 field results in phosphorylation of MAPK/ERK[29]. Furthermore, NMDA receptor stimulation in hippocampal slices results in ERK2 activation. Finally, inhibition of ERK with SL327 reduced long-term spatial learning in a Morris water maze task in mice[30]. These findings support the key role of MAPK/ERK in L-LTP and LTM[23,29] .

The MAPK/ERK cascade consists of three types of kinases. The MAPK kinase kinase (MAPKKK) family, which includes Raf1 and B-Raf kinases, activates MEK through serine/threonine phosphorylation. Following this, MEK phosphorylates both a threonine and a tyrosine residue and activates MAPK. The MAPK family consists of p44 MAPK (ERK1) and p42MAPK (ERK2)[29].

It seems that there are several mechanisms for stimulation of ERK/MAPK pathway (Figure 4). First of all, Ca^{2+} activates CaM that, in turn, stimulates AC1 and AC8. As mentioned earlier, DA can also activate ACs. Following this, AC1 and AC8 elevate cAMP levels in the postsynaptic cell. Increases in cAMP stimulate guanine nucleotide exchange factors (EPAC) for the Ras-related proteins 1 (RAP1) and 2 (RAP2). EPAC triggers MAPK/ERK cascade by activating RAP1 that, in turn, stimulates Raf kinases[23,29].

A second pathway is through the direct stimulation of guanylyl nucleotide-releasing factor (RasGRF1) by either Ca^{2+} or CaM. RasGRF1 activates Ras that triggers the activation of the MAPK/ERK cascade[23,29].

Finally, a third pathway is through cAMP and PKA. AC-induced increases in cAMP levels result in PKA activation, which stimulates sequentially RAP1 and MAPK/ERK[23]. Therefore, the MAPK/ERK pathway may be another convergence point that integrates signals from different pathways (Figure 4). Both CaM-mediated and AC/cAMP-mediated activity can activate this pathway[23,29].

ERK also appears to have an effect on E-LTP. Two substrates of ERK are the voltage-dependent potassium channel Kv4.2 and synapsin I. Decreased activity of Kv4.2 receptors results in increased neuronal excitability and a potentiated LTP. Additionally, the phosphorylation of synapsin I by ERK mobilizes vesicles towards the active zone of the presynaptic neuron, which facilitates neurotransmitter release[13,29].

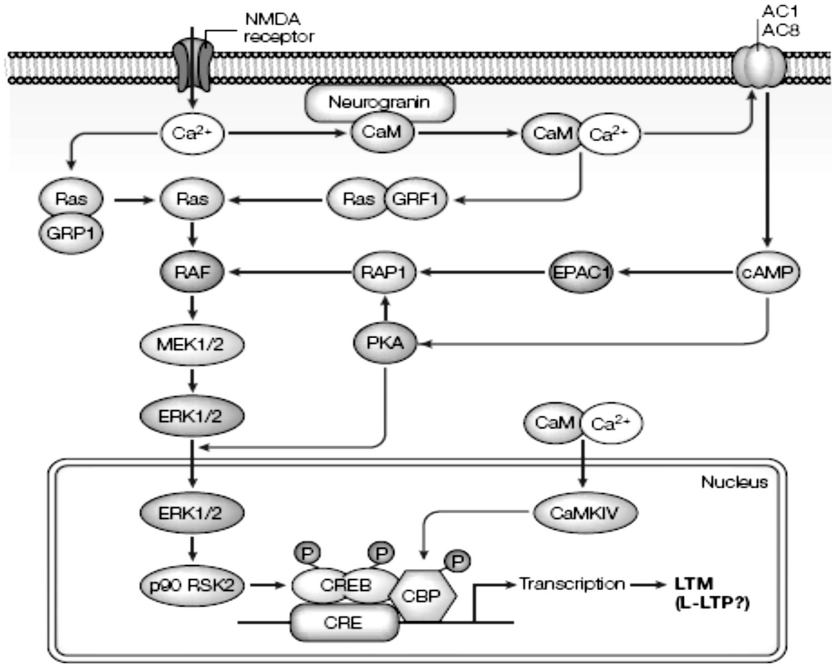

Figure 4. Different molecular pathways lead to MAPK/ERK activation in L-LTP. The Ras family plays a crucial role in MAPK/ERK activation and CREB phosphorylation (Xia and Storm, 2005, Nature Reviews).

CREB as a convergence point

ERK phosphorylates CREB indirectly via phosphorylation of the ribosomal protein S6 kinase (RSK2)[13,23,29,31]. However, ERK has to translocate to the nucleus to phosphorylate RSK2, a process that requires activation of PKA[23] (Figure 4). This may be another mechanism by which PKA is involved in L-LTP and CREB-dependent transcription. RSK2 phosphorylates CREB at Ser133, which is followed by CRE-mediated transcription and the recruitment of the CREB-binding protein (CBP). The CREB/CBP complex, in turn, phosphorylates downstream TFs and initiates transcription[31].

There is plenty of evidence that emphasizes the role of CREB in L-LTP and LTM. First of all, transgenic mice lacking the α and Δ isoforms of CREB show impaired L-LTP in the CA1 region of the hippocampus. Moreover, the $CREB^{\alpha\Delta-}$ mutants were impaired in long-term hippocampus-dependent memory[32]. Finally, examination of CREB activity in a strain of mice, after insertion of the CRE-driven b-galactoside gene in their genome, demonstrated that LTP induction increased CREB phosphorylation in the field CA1 of the hippocampus[33].

Ca^{2+} entry also results in an early and transient phase of CREB phosphorylation. Calcium/calmodulin-dependent kinase IV (CaM-KIV), a nuclear kinase, triggers the initiation of this phase[23,31,34]. CaM-KIV phosphorylates CREB directly at Ser133[24,31]. The time course of CaM-KIV expression in the nucleus correlates positively with the appearance of phosphorylated CREB in hippocampal slices[35]. Moreover, inhibition of CaM-KIV with antisense oligonucleotides impairs CREB phosphorylation and CRE-dependent transcription[34,36]. Furthermore, transgenic mice with a dominant-negative form of CaM-KIV (dnCaM-KIV) in their forebrain had impaired L-LTP in the field CA1 of the hippocampus[34]. Additionally, levels of CREB and CRE-dependent transcription were reduced in the hippocampus of these mutants. Finally, the consolidation phase of hippocampus-dependent memory was impaired[34].

Collectively, these data indicate that CaM-KIV is an independent signal transduction pathway of the CREB/CRE-mediated transcription in the hippocampus and is important for LTM[23] (Figure 4). It may be that CREB phosphorylation can be stimulated by both pathways; the fast but transient CaM-KIV pathway,

and the slower but more persistent MAPK/ERK pathway[34].

Most important, however, CREB appears to be another convergence point where Ca^{2+}-induced signals are integrated and participate in gene transcription and protein synthesis. Although CRE-mediated transcription in LTP is a complex phenomenon that depends on the involvement and interaction of many molecular pathways, it appears that activation of CRE-mediated transcription depends on Ca^{2+} and is mediated by at least four enzymes: Ca^{2+}/CaM, AC, MAPK/ERK, and CaM-KIV[23].

CONCLUSIONS

The firm conclusion derived from this review could be that both implicit and explicit memory in invertebrates and mammals share common molecular mechanisms. First of all, different kinds of learning and memory are represented in the CNS of a variety of species in the form of strengthened synapses. Furthermore, calcium and the AC/cAMP cascade are essential components of the early and late phases of memory in both invertebrates and mammals. Moreover, protein synthesis and mRNA translation characterize the late phase of implicit and explicit memory and CREB plays a key role in it. As Kandel states "some aspects of implicit memory storage in invertebrates have been conserved over millions of years of evolutionary time in the mechanism by which explicit memory is stored in vertebrates" [4].

Despite the similarities, however, the molecular mechanisms of explicit memory in mammals are more complicated than those underlying implicit memory in invertebrates. This can be confirmed from the existence of many convergence points in LTP that integrate parallel molecular signals and transmit the information to downstream substrates. The MAPK/ERK pathway is an example of such a convergence point in LTP. Moreover, it seems that there are independent parallel pathways that activate gene expression and each of them may transmit different types of information at different temporal phases, thus, exerting different effects. An example is the parallel activation of the CaM-KIV and MAPK/ERK pathways that both lead to the phosphorylation of CREB at different temporal points.

The discovery of the molecular components of memory opens the way for the development of new drugs and more effective treatments for many psychiatric and neurological diseases. Alzheimer's dementia and Post-traumatic Stress Disorder (PTSD) are two prominent examples and people suffering from these disorders could benefit from this kind of research. Moreover, new memory-enhancing medications could help healthy people, for example, the elderly who face some normal age-related memory impairment. Despite the ethical dilemmas that will follow the development of this molecular technology (who is going to use it, when, and especially why; what kind of side effects will follow this medication), it is widely believed that it will improve the quality of life of many people in the future.

Μοριακοί μηχανισμοί της μνήμης και της μάθησης

Δωροθέα Καπουκρανίδου, Χαρίλαος Παπαχρήστου, Επαμεινώνδας Γουσόπουλος

Εργαστήριο Φυσιολογίας, Ιατρικής Σχολής Α.Π.Θ.

ΠΕΡΙΛΗΨΗ: Σκοπός της παρούσας εργασίας είναι η μελέτη των μοριακών μηχανισμών της μνήμης και της μάθησης. Το πρώτο μέρος της εργασίας ερευνά τους μηχανισμούς της άδηλης μνήμης στο θαλάσσιο σαλιγκάρι Aplysia που μελετήθηκε διεξοδικά από τον διεθνούς φήμης και κάτοχο του βραβείου Νόμπελ ερευνητή Eric Kandel. Η άδηλη μνήμη υποδιαιρείται σε δύο στάδια με γνώμονα την χρονική διάρκειά της: την βραχύχρονη και την μακρόχρονη μνήμη. Οι μοριακοί μηχανισμοί του κάθε σταδίου θα εξεταστούν σε χωριστές ενότητες.
Το δεύτερο μέρος ερευνά τον κυτταρικό μηχανισμό της συνειρμικής μακρόχρονης ενδυνάμωσης στην περιοχή του ιππόκαμπου που θεωρείται ότι παίζει καθοριστικό ρόλο στην έκδηλη μνήμη των θηλαστικών. Όπως και η άδηλη μνήμη στην Aplysia, έτσι και η έκδηλη μνήμη στα θηλαστικά διαιρείται σε βραχύχρονη και μακρόχρονη.

Λέξεις Κλειδιά: Μνήμη, Μάθηση, Μοριακοί μηχανισμοί, NMDA υποδοχείς.

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Abbreviations

***AC**, adenylate cyclase*
***AMPA**, α-amino-3-hydroxy-5-methyl-isoxazolepropionic acid*
***AP5**, 2-amino-5-phosphonopentanoic acid*
***Ca**, calcium*
***CA1**, cornu ammonis 1*
***CaM**, calmodulin*
***CaM-KII**, calcium/calmodulin-dependent kinase Type II*
***CaM-KIV**, calcium/calmodulin kinase IV*
***cAMP**, cyclic adenosine monophosphate*
***CPEB**, cytoplasmic polyadenylation element-binding protein*
***CRE**, cAMP response element DNA sequence*
***CREB**, cyclic adenosine monophosphate response element binding protein*
***CS**, conditional stimulus*
***DA**, dopamine*
***DG**, dentate gyrus*
***E-LTP**, early phase of LTP*
***EPSP**, excitatory postsynaptic potential*
***GluR1**, glutamate receptor subunit 1*
***5-HT**, serotonin*
***L-LTP**, late phase of LTP*
***LTM**, long-term memory*
***LTP**, long-term potentiation*
***MAPK**, mitogen-activated protein kinase*
***MAPKKK**, mitogen-activated protein kinase kinase kinase*
***MEK**, MAPK kinase*
***NMDA**, N-methyl-D-aspartate*
***NR1**, N-methyl-D-aspartate subunit 1*
***PKA**, protein kinase A*
***PKC**, protein kinase C*
***PP1**, protein phosphatase 1*
***RAP**, Ras-related protein 1*
***Ras**, family of guanine trinucleotide binding protein (GTP) hydrolases*
***RSK**, ribosomal S6 kinase*
***STM**, short-term memory*
***TF**, transcription factor*
***US**, unconditional stimulus*