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Molecular Basis of Learning and Memory

Molecular Alterations in Learning and Memory

Perçin PAZARCI[1*]

Abstract:

As commonly known, learning is the process of acquiring a new information and memory is preservation of acquired information for later use. The difference in learning and memory capacities between different species and between different individuals of same species directed scientists to research the causes of this. According to commonly accepted approach these differences are due to the distinctions in synaptic alterations. In collaboration with advancing molecular techniques, the formation mechanisms of synaptic alterations, the reasons of differences between them, the changes that occur in neurons during learning and the changes that occur in neurons as a result of memory became popular research subjects nowadays. The researches that has been done are insufficient, however, many important findings are obtained until now. The changes that occur in pre-synaptic and post-synaptic neurons during simple non-associative learning, associative learning, short term memory and long term memory have been researched and different molecular mechanisms have been suggested.

Keywords: Non-Associative Learning, Associative Learning, Memory, Long Term Potentiation, Synaptic Plasticity

[1] Dr. Perçin PAZARCI: Çukurova Üniversitesi Tıp Fakültesi, Tıbbi Biyoloji Anabilim Dalı, Sarıçam, Adana, Türkiye

e-mail: ppazarci@cu.edu.tr

ORCID iD: 0000-0002-3708-0054

INTRODUCTION

In the world of neuroscience, one of the most complex and fascinating mysteries is the process of learning and memory. From the first steps taken by a newborn baby to the vivid memory of valuable experiences in old age, the brain's ability to acquire, store and recall information is truly remarkable. Over the years, scientists have delved deeply into the neural underpinnings of this phenomenon, uncovering a complex molecular fabric that brings together the essence of learning and memory.

Although there are many different definitions of learning and memory made by different branches of science such as psychology, sociology, neurology, if we put these definitions together in the simplest form, learning is the act of acquiring new knowledge (or ability, behavior, preferences, values, etc.), and memory is the storage of this information permanently to be used later when necessary. The capacity to learn and remember what has been learned is the biggest feature that makes humans different from other living creatures. Just as there are differences in the capacity to learn and remember among different species, the fact that there are also differences between individuals of the same species has led scientists to search for the reasons that create these differences. Especially in recent years, with the advances in molecular techniques, previous neurology and psychology studies have gained a different dimension, and the molecular mechanisms that play a role in the ability to learn and remember have become an important research area.

Memory and learning were defined as a result of synaptic alterations by Hebb (1), who carried out very important studies in the field of neuropsychology. Today, although this idea is still accepted to some extent, it is argued that learning and memory are not only a result of these alterations, but that many mechanisms within neurons are also very important in these two events(2). Research on the second idea has generally intensified after the 1980s, and studies have been conducted on many different model organisms. Among these studies, Kandel and Schwartz (3) and Carew et al (4) can be considered the most important and pioneering ones. Due to its ability to perform specific forms of learning (habituation, sensitization, conditioning), the simplicity of its nervous system, the ease of deciphering its genetic structure, and the presence of large neurons, Aplysia californica (sea slug) is currently the most commonly chosen model organism in research on learning and memory(5).

Learning

Learning styles in living things can be divided into many subcategories such as non-associative learning, associative learning, imitation, learning by watching and listening, and learning through experience. Since most of these categories are the areas of interest of psychology and neurology, this article will focus on non-associative learning and associated learning, which are the basic learning styles on which molecular research focuses. The reason why molecular research focuses on these two subunits is that the results cover one or a few neurons and their results give an idea about the functioning of the entire system.

Non-Associative Learning

Non-associative learning can be defined as making an inference directly from a situation without directly associating it with another event(6). This learning form is examined in two subcategories: habituation and sensitization.

Habituation is the state in which the response in animals decreases as they are exposed to a repetitive stimulus for a certain period of time(7). It is a different form of integration. If an animal is exposed to a stimulus and reacts, but later realizes that this stimulus is not harmful or beneficial, it concludes that the stimulus is meaningless and its response gradually decreases with repeated stimuli, or its response may even stop completely. A simple example of this is the behavior of small birds. When these birds see a large predator bird placed in a cage, they reflexively start to run away. However, if the stimulus is repeated in the same way, it realizes that the predator cannot reach it and stops its reaction.

Sensitization is the sudden increase in the response to a repeated stimulus above the normal level or an increase in the response to the same stimulus after habituation, typically by changing the stimulus(7). Using the example given above, if the predator bird is released from its cage, the prey bird's response will be greater than usual because the stimulus has suddenly changed, catching the prey bird unprepared. Another example of sensitization could be a person rubbing his arm. The first reaction this person will give will be the same as the reaction he would give to the slight warmth he would feel. However, if the stimulus (rubbing) continues to be repeated, the warmth may start to be perceived as pain, leading to a change in the response.

These two simple forms of learning have been observed in all living things with a nervous system and have molecularly the same dynamics(8). Kandel and Schwartz (3) first explained the molecular mechanism and formation of these learning styles with their research. In their study, the behavior of Aplysia californica was examined. This organism has a structure called a siphon, and it possesses sensor neurons connected to this region (Figure 1)(9). These sensor neurons are linked to motor neurons that control the gill muscles. When the siphon is touched or stimulated, the interaction of these neurons results in the organism retracting its gill through a coordinated response (gill withdrawal reflex). In the conducted study, the contraction potential of the gill muscles was measured in response to the initial stimulation of the siphon and with an increasing number of stimuli. It was observed that this potential gradually decreased with each subsequent stimulation (habituation)(3). Following the habituation phase, the organism's head region was subjected to an electric shock, and then, when the siphon was touched, the contraction potential of the gill muscles was measured. Interestingly, it was observed that this potential was significantly higher than the normal potential (sensitization)(3). Following this stage, the question "What triggers these different responses?" arose, leading to an investigation of the changes occurring in neurons after habituation and sensitization. During the first and next few touches to the siphon, neurons generate action potentials in a normal manner. However, as the number of stimulation increases, calcium channels at the axon ends of pre-synaptic neurons (sensor neurons) are partially inactivated and the amount of neurotransmitter substance secreted to post-synaptic neurons (motor neurons) decreases. As the number of stimuli increases, more calcium channels become inactivated, resulting in a decrease in the response. Even when there is a break of several hours in touching the siphon, very little response is elicited from the organism upon subsequent stimulation(3). This has been considered as the simplest learning dynamic.

In the next phase of the study, an electric shock was given to the head area of the organism. It was found that this shock stimulated the third group of neurons seen in Figure 1 and triggered the release of serotonin from these neurons(3). As shown in Figure 2(10), it was found that the released serotonin subsequently triggered cAMP synthesis in sensor neurons, and cAMP activated protein kinases. The activated protein kinases then inhibited serotonin-sensitive potassium channels, reducing their activity. As a result, due to the potassium channels responsible for repolarization in neurons, the decreased activity leads to prolonged action potential duration. Consequently, it was concluded that the calcium channels at the axon terminals remained open for a longer duration, resulting in an increased release of neurotransmitter from sensor neurons(3). As one might predict, the increased neurotransmitter amount will lead to more stimulation in the post-synaptic region and confirm the observed situation. In many subsequent studies with different organisms, similar results have been found, and it is generally accepted that basic forms non-associative learning are consistent across various species(11).

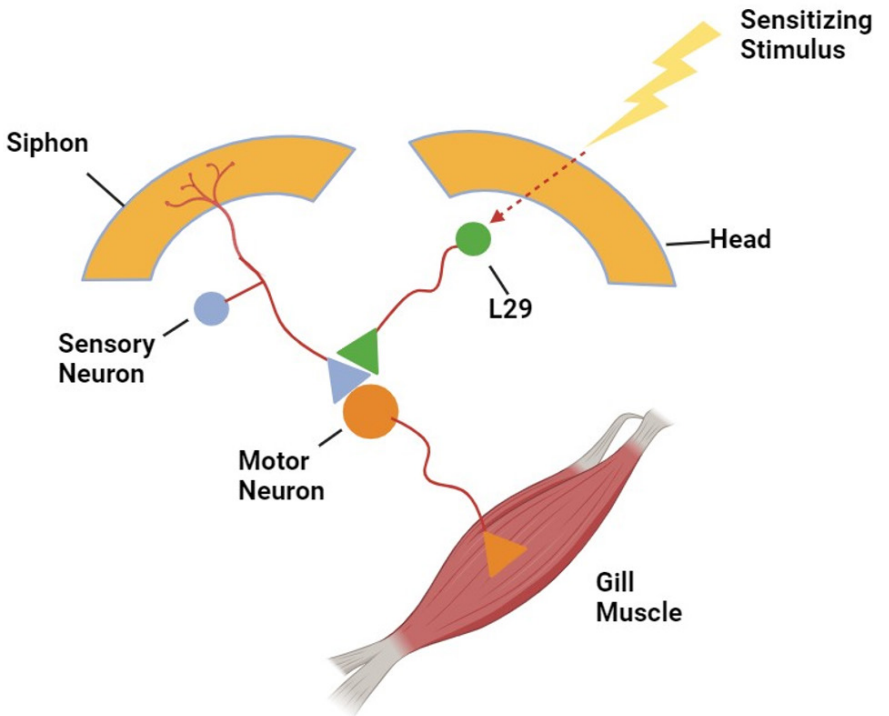

Figure 1. Representation of the nervous system of Aplysia California

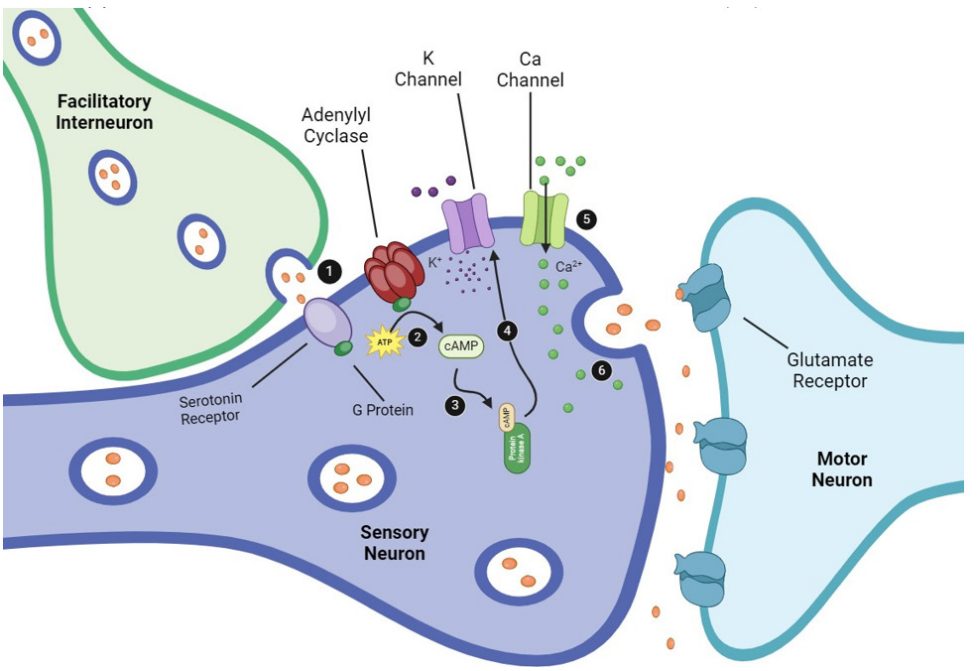

Figure 2. Short-term sensitization. **(1)** Binding of serotonin to G-Protein coupled receptor. **(2)** Stimulation of cAMP formation. **(3)** Binding of cAMP to the regulatory subunit of Protein Kinase A (PKA). **(4)** Catalytic subunits of PKA phosphorylate potassium channels. **(5)** Closing of potassium channels, prolongation of action potential duration, more calcium inflow. **(6)** More neurotransmitter release as a result of excess calcium(10).

In studies conducted in subsequent years, it has been frequently claimed that habituation and sensitization are not only short-term but also lead to long-term learning(12). Various research has been conducted on this subject. Dash et al (13) proposed, contrary to the mechanism described above that remains active for a short period, that for this simple form of learning to be long-lasting and transform into a kind of memory, some form of protein synthesis is necessary. In their research on this subject, they hypothesized that cAMP not only activates protein kinases but also binds to Cyclic AMP Response Element-Binding proteins (CREB). As it is known, CREBs perform functions that regulate transcription in the cell. The results of their research indicated that the activation of CREBs leads to changes in the number of potassium channels sensitive to various neurotransmitters and voltage-gated calcium channels. They have suggested that this plays a role in the permanence of learning(13). In subsequent similar studies, it was observed that organisms given CREB inhibitors during habituation or sensitization were unable to convert this short-term learning into long-term learning(14). This observation supported the idea that CREB plays a crucial role in the conversion of short-term into long-term learning.

Associative Learning

Associative learning can be categorized into classical conditioning and operant conditioning. Classical conditioning is defined as the triggering of one stimulus by another independent stimulus when they are perceived consecutively for a long time. It was first discovered by Ivan Pavlov as a result of his research on dogs.

Operant conditioning is the regulation of subsequent behaviors according to the consequences of a behavior. This concept was initially introduced by Edward L. Thorndike. The increased permanence of these two types of learning and the greater resemblance of the mechanisms influencing long-term memory compared to non-associative learning have intensified interest in this subject. Kandel (15) demonstrated that Aplysia californica can learn through classical conditioning and explained changes at the molecular level. In the conducted study, the snails were first subjected to an electric shock to the head region, followed by touching their siphon. As previously indicated, an increased response in the gill withdrawal reflex was observed compared to normal. A second group of Aplysia was not subjected to electric shock and served as the control group. It was found that, after a certain period the snails in the first group exhibited a heightened response when their siphon was touched even without receiving electric shock, compared to the control group. Furthermore, this heightened response could extend for up to 3-4 weeks depending on the frequency of electric shock application. Subsequently, the neurons leading to the sensor neuron from the head region of the snails that received electric shocks were destroyed, after which their siphon was touched. Surprisingly, it was measured that the organisms exhibited the same intensity of reflex(15). This led to the conclusion that the changes were not due to alterations in the neurons originating from the head region involved in conditioning, but rather resulted from changes in the neurons originating from the siphon. These changes were then investigated. It was found that repeated electric shocks increased serotonin release from neurons originating from the head region, leading to longer opening of calcium channels. However, with the continuous repetition of electric shocks, it was observed that the calcium levels in neurons increased significantly. Due to the excess calcium levels, some of the calcium binds to calmodulin. The resulting complex was observed to activate a type of adenylate cyclase called calcium-calmodulin-dependent adenylate cyclase, which is found exclusively in neurons. It has been suggested that instead of the short-lived activation via G-protein seen in classical sensitization, the stable structure of the calcium-calmodulin complex triggers the synthesis of cAMP in the cell for a prolonged period, thereby ensuring that the

conditioning is not forgotten for a certain period of time(15). This study, while not explaining situations where conditioning lasts for an extended period, has shed light on medium-term memory in organisms.

Memory

Memory is generally studied under two main categories: short-term and long-term memory. Short-term memory involves the immediate processing and evaluation of stimuli from sensory organs, followed by its rapid decay. The molecular dynamics of short-term memory are generally accepted to be similar to those of simple non-associative learning, as described earlier. Since necessary information about this topic has been provided above, this section will focus on long-term memory.

According to neuroscience, synaptic alterations form the basis of all memory, as mentioned earlier. These alterations include Long-Term Potentiation (LTP), Long-Term Depression (LTD), and Synaptic Plasticity (SP)(16). Briefly, LTP refers to the long-lasting and persistent enhancement of communication between two neurons that are continuously stimulated together. LTD, on the other hand, is the opposite, involving a long-lasting decrease in the connection between two neurons. SP encompasses the ability of synapses between two neurons to change their interaction depending on the current situation(16). While studies in neuroscience have revealed many findings about which brain regions are active during specific activities, they have not fully explained the reasons behind LTP, LTD, and SP occurring in different brain regions. To elucidate these reasons, numerous studies are ongoing, utilizing advancing molecular techniques. The aim of these studies is to uncover the molecular mechanisms underlying long-term learning and memory.

Two different perspectives have been proposed for molecular bases of these changes. The first one suggests that synaptic function changes as a result of modifications in synaptic proteins. Shi et al (17) found that N-methyl D-aspartate receptors (NMDAR) and amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) in the postsynaptic neuron are activated by a type of protein kinase called continuous kinases. NMDAR and AMPAR are ionotropic glutamate receptors, and when glutamate binds to these receptors, they allow the entry of cations such as calcium, sodium, and potassium into the cell(10). According to their findings, an enzyme called ubiquitin hydrolase is produced in the postsynaptic neuron that is continuously stimulated together, and the amount of ubiquitin in the postsynaptic region is reduced in this way. Ubiquitin is known to mark proteins for degradation, and as a result of its decreased amount, the duration of activity of proteins in the postsynaptic region is prolonged. Consequently, continuous kinases emerge, and the activities and permeabilities of NMDAR and AMPAR change. When this situation persists, SP and LTP occur(17).

According to the second proposed model, the strength of the synapse between two neurons is dependent on the number of ion channels it possesses(18). In a dynamic state, NMDAR and AMPAR are continuously balanced through exocytosis and endocytosis. However, changes in synaptic activity between two neurons can shift this balance towards the addition or removal of new NMDAR and AMPAR, resulting in either LTP or LTD(19). Additionally, in LTP, it has been determined in various studies that these types of receptors outside the synaptic region are encouraged to undergo endocytosis by dynamin and clathrin and are subsequently transported to the synaptic region(20).

Hawkins et al (21) investigated the changes occurring in the presynaptic neuron during LTP and SP through their research. They suggested a model where a series of activations and protein syntheses work together and influence long-term memory. The proposed model resulting from this study is depicted in Figure 3(22).

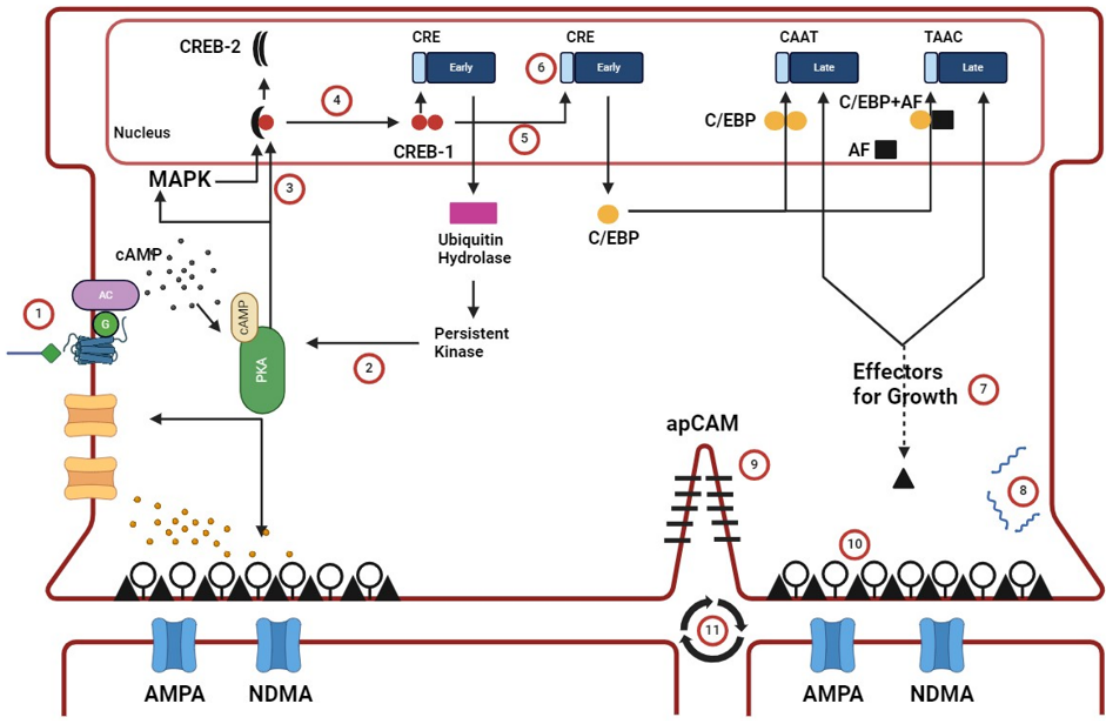

Figure 3. Changes in the pre-synaptic neuron in LTP and SP. **(1)** Short-term strengthening of synaptic connection as a result of neurotransmitters. **(2)** Balance of kinase and phosphatase activities at the synapse. **(3)** Retrograde transport from synapse to nucleus. **(4)** Activation of transcription factors. **(5)** Activity-dependent induction of gene expression. **(6)** Chromatin alteration and epigenetic changes. **(7)** Capture of newly synthesized products by synapses. **(8)** Stimulation of local protein synthesis in active synapses **(9)** Development of the synapse and formation of new synaptic regions. **(10)** Activation of pre-existing silent synaptic regions. **(11)** Stimulation of other nearby neurons by self-triggering molecular mechanisms(22).

In conclusion, studies on long-term memory have proposed various models, but there is no definitive consensus on how learned information is stored in the brain for extended periods or how it is recalled when needed. Many studies have found differences in learning and memory potential as a result of the stimulation or inhibition of various receptors or kinases with certain chemicals. However, these findings are still insufficient to explain how higher-level organisms perform complex brain activities such as memory recall, thinking, or learning from experiences.

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