Project Group 2

The Molecular Basis for Long-Term Memory: Protein Synthesis Versus Protein Modification

The prevailing current view on long-term memory is activity dependent synaptic protein synthesis is necessary for memory storage and retrieval. This idea is most strongly advocated by Dr. Eric Kandel, and we have included two papers by him that explain this model. In his 2001 review, Kandel overviews his ideas on how new protein synthesis affects synapses, thus creating and modifying memories while a more specific experimental illustration is provided by Abel et al.

Challenging the model that protein synthesis is solely responsible for long-term memory, Routtenberg and Rekart propose that protein modification is sufficient. Their ideas are outlined in their 2005 review while Pang et al. provide specific evidence for post-translational protein alterations.

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Some history on the study of memory...

Any brief history given on studies of memory would have to include Eric Kandel, a neuroscientist at Columbia who was and continues to be involved in many prominent studies involving learning and memory.

In 1958, Kandel & Spencer started studying the hippocampus, which is the part of the mammalian brain thought to be most directly involved in aspects of complex memory. They began their study logically but at what could be considered its most complex form by asking if the electrophysiological properties of hippocampal pyramidal cells (which are thought to be the key hippocampal cells involved in memory storage) are somehow different from other neurons in the brain. The answer they found was no—all nerve cells actually have similar signaling properties. So this means that intrinsic signaling properties themselves do not give any key insight into memory storage.

As another approach, Kandel next attempted a radical reductionist approach where he chose to study the simplest instances of memory storage...
Aplysia Model

The simplest usable model for memory that Kandel and his colleagues could find was the Aplysia, the giant marine snail. This organism was used for several reasons: its nervous system has a relatively small number of cells, and many of these are gigantic and uniquely identifiable.

In studying the Aplysia, a very simple defensive reflex was found, called the gill withdrawal reflex, where the gill and siphon withdraw upon strong stimulation of the tail (much like the quick withdrawal of a hand from a hot object). They found that this reflex could be modified by 3 forms of learning (which correspond to 3 different types of learning in humans): habituation, sensitization, and classical conditioning.

Initially, Kandel and his colleagues focused specifically on one type of learning: sensitization, which is basically a form of learned fear in which the animal learns to respond strongly to an otherwise neutral stimulus. In order for this to happen, the aplysia gets an aversive shock to the tail, it recognizes the stimulus as aversive, and learns to adapt its reflex responses to subsequent stimuli, even when they are harmless. This type of sensitization can be likened to a person hearing a gunshot, and subsequently being easily startled (by a tap on the shoulder, for instance).

In order for this type of sensitization to occur, an animal must remember a previous aversive stimulus, and they find that how long that memory lasts is a function of the number of repetitions of the stimulus. For instance, a single shock causes a memory that lasts only minutes, and does not require the synthesis of new protein, whereas multiple spaced shocks to the tail cause memory that lasts much longer, and does require protein synthesis.

Overall, these findings support the idea that Aplysia learning, like vertebrate learning, has 2 stages: transient memory which lasts minutes, and enduring memory which can last much longer.

Protein Synthesis Model for Long-term Memory

These findings from the Aplysia studies along with others helped shaped the current model of short and long term memory. It is thought that short term synaptic changes involve modification of proteins which then modify synaptic connections, while long term synaptic changes involve new protein synthesis, the formation of new connections, and activation of gene expression.

It is found that stimuli that cause short-term change "mark" the synapse for later long-term change, but local protein synthesis is needed for structural changes to persist because it continually draws proteins to recently activated synapses, which causes the change to be permanent. Basically, it is the network formed by these stabilized synapses that is thought to serve as the neural representation of the long-lasting memory.


The specific research paper that we looked at which supports the protein synthesis theory was done by Abel et al. in 1997. They showed the importance of PKA activity in the late phase of LTP. In order to do this they used transgenic mice that express a mutant form of PKA, called R(AB). The reduced PKA activity caused a decrease in late-phase, but not early-phase LTP, which supports the idea that the activity of this protein is necessary just for late-phase LTP. They also found that the deficit in late-phase LTP was paralleled by a deficit in long-term memory, R(AB). The reduced PKA activity caused a decrease in late-phase, but not early-phase LTP, which supports the idea that the activity of this protein is necessary just for late-phase LTP. In order to do this they used transgenic mice that express a mutant form of PKA, called R(AB). The reduced PKA activity caused a decrease in late-phase, but not early-phase LTP, which supports the idea that the activity of this protein is necessary just for late-phase LTP. In order for this type of sensitization to occur, an animal must remember a previous aversive stimulus, and they find that how long that memory lasts is a function of the number of repetitions of the stimulus. For instance, a single shock causes a memory that lasts only minutes, and does not require the synthesis of new protein, whereas multiple spaced shocks to the tail cause memory that lasts much longer, and does require protein synthesis.

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Figure 1: A simple learned behavior. (A) A dorsal view of Aplysia showing the gill, the animal's respiratory organ. A light touch to the siphon with a fine probe causes the siphon to contract and the gill to withdraw. Here, the mantle shelf is retracted for a better view of the gill. Sensitization of the gill-withdrawal reflex, by applying a noxious stimulus to another part of the body, such as the tail, enhances the withdrawal reflex of both the siphon and the gill. (B) Spaced repetition converts short-term memory into long-term memory in Aplysia. Before sensitization training, a weak touch to the siphon causes only a weak, brief siphon and gill withdrawal reflex. Following a single noxious, sensitizing shock to the tail, that same weak touch produces a much larger siphon and gill reflex withdrawal response, an enhancement that lasts about 1 hour. More tail shocks increase the size and duration of the response (Kandel, 2001).

Figure 2: (A) The protocol for context conditioning consists of exposure to the context followed by a tone and then a shock. The animals are then tested 1 hour and 24 hours after training. (B1) Mutant mice that express the R(AB) gene in the hippocampus, blocking the action of PKA, have a selective defect for long-term contextual memory. Mice that express R(AB) were conditioned to freeze to the context. After becoming familiar with the context, the mice heard a sound and received a shock through the electrified grid in the floor. As a result the animals learned to associate the context of the space with shock and to freeze when placed in the box at a future time. These mice had good short-term memory at 1 hour for freezing to context, but at 24 hours they no longer froze to context, indicating a defect in the form of long-term explicit (declarative) memory that requires the hippocampus. (B2) Wild-type mice exposed to anisomycin, an inhibitor of protein synthesis, during training show a similar defect for long-term memory when tested 24 hours after conditioning. (C) Place cell stability for R(AB) and wild-type mice. R(AB) mice with a defect in PKA and late LTP form place fields that are stable at 1 hour. These fields are not stable at 24 hours (Kandel, 2001).

For a more detailed examination of the findings of this paper, click here.
Potential concerns with the Protein Synthesis Model...

1. There are actually many instances of long-term memory occurring while protein synthesis was being inhibited. For instance, long-term olfactory memories and those for color discrimination learning in honeybees have been shown to form normally in the presence of over 90% protein synthesis inhibition. Furthermore, normal retention of memories has also shown to occur while synthesis is being inhibited just by modifying training procedures (such as increasing foot shock intensity).

2. Numerous substances which are able to rescue memory from protein synthesis inhibition, without actually reversing the inhibition. These substances include amphetamines, caffeine, fluoxetine and nicotine.

3. PSIs might actually increase protein synthesis themselves through a dominant-negative effect. This is the idea that protein synthesis inhibitors might inhibit negative regulators of gene expression such as suppressors or silencers and in doing this actually increase protein levels.

4. Possible effects of a 'dirty drug' It is typical to conclude that protein synthesis inhibition is the cause behind impaired memory when you are using PSIs, but it has been found that these inhibitors can also have other side effects. For instance, the PSI anisomycin actually also causes post-translational modification of proteins and inhibits catecholamine function, raising the question of whether one of these effects might actually be responsible for mediating the storage of long-term memory.

Due to these potential side effects of PSIs, and because there are enough instances of memory storage in the virtual absence of protein synthesis, alternative models must be considered...

Protein Modification: A new theory enters the picture

In 2005, Routtenberg & Rekart at Northwestern published a new theory on long-term memory storage in the journal Trends in Neuroscience. They agree that it is the synapse that is modified in response to learning-associated activity (an idea first advocated by Ramon y Cajal a century ago), but the key difference with their proposed theory is the emphasis they place on post-translational modification (or PTMs). Their argument is that learning leads to a post-translational modification of a synaptic protein that results in changes to shape, activity and/or location of existing synaptic proteins. In this model, PTM of synaptic proteins is the only mechanism required for long-term memory.

The novel feature of this proposed model is that endogenous brain activity (or non-random spontaneous activity) works as a positive-feedback rehearsal mechanism which updates network by fine-tuning the PTM state of previously modified proteins. It is thought that this continual updating is responsible for allowing long-term memory to occur.

In many ways this new theory avoids many concerns of the current model. For instance, it explains how long-term memory storage could occur while protein synthesis is being inhibited. Unlike the current theory, the new theory argues that the changes underlying memory are not stable at the level of the synapse, but are rather "meta-stable" at the level of the network. "Meta-stable" refers to the fact that the network is not permanent, but relatively long-lasting. This can be considered a more dynamic view of memory storage than the the current prevailing view.

Although current views on long-term memory storage are unclear as to whether one or multiple neural networks are involved in representing each memory, the new proposal by Routtenberg & Rekart advocates that each memory corresponds to multiple neural representations. In accordance with this idea, the reason it is hard for someone to forget their own name, for instance, is not because it is associated with permanent changes in certain synapses, but rather because memories are re-duplicated in many different networks. This re-duplication makes forgetting unlikely; however, it is possible considering that each component of each network is transient and malleable. This type of drastic amnesia is seen in the advanced stages of Alzheimer's Disease, for instance.
More about Post-Translational Modifications (PTMs):

A group of PTMs that affect neuronal plasticity regulate synapse efficacy in response to a learning event. The new model advocates that the continual modification of PTMs is the mechanism that allows for memory to be maintained long-term. PTMs are regulated by proteases, kinases, and phosphatases. Some example protein modifications that are thought to be able to mediate long-lasting synaptic change are autophosphorylation of protein kinase, proteolytic cleavage, and actin polymerization. The protein kinase autophosphorylation PTM mechanism is proposed to maintain the activation state of kinase activation state. The function of proteolytic cleavage is to expose cryptic glutamate receptors or the catalytic subunit of kinases, and actin polymerization serves to alter synaptic cytoskeleton. While these are just a few examples of potential post-synaptic modifications that are thought to maintain long-term change, it is the entire group of PTMs that respond to learning together that are responsible for maintaining memory.

More about Endogneous Activity:

As mentioned above, after PTMs allow synaptic change in response to learning, it is actually the continual updating of these modified states which allows the memory to last long-term. The mechanism that allows for this continual updating or fine-tuning of the previously modified proteins is endogenous activity, or non-random spontaneous activity of the brain. This endogenous activity essentially causes a positive-feedback rehearsal mechanism responsible for the continual updating of previous modifications.

Since this positive feedback system is essentially self-sustaining, it also has many built-in control mechanisms (such as endogenous kinase inhibitors and mechanisms of synaptic inhibition) that prevent runaway feedback.

There is increasing evidence that rehearsal might actually be present during sleep, and can directly correlate to activity patterns generated during learning. However, whether this rehearsal occurs during sleep or wake, these repeating patterns of neural activity would be significant evidence for the maintenance of PTMs brought about by original learning.
What about protein synthesis?

With this new theory that places great emphasis on post-translational modification, one might wonder, "what, if anything, is the role of protein synthesis in this new model?" Routtenberg and Rekart argue that protein synthesis is not unimportant in their model, but it is exclusively a permissive, replenishment step that could serve to replenish proteins during the rapid turnover that occurs during post-translational modification. While the role of protein synthesis is to replenish, it is only the post-translational modifications which are able to bring about synaptic change.

Experimental Evidence for the New PTM Model (Pang, 2004):

This paper is significant because it highlights the many problems associated with current views of long-term memory, and advocates the need for an alternative model. It includes several lines of experimental evidence which support the new theory. First, it identifies a key PTM mechanism (proteolytic cleavage) that they find to be crucial in leading to late-phase LTP. They also experimentally illustrate that LTP blocked by protein synthesis inhibitors (PSIs) can be rescued without reversing protein synthesis inhibition.

For a more detailed examination of the findings of this paper click here.

Keeping It All in Perspective...

In assessing this new theory for long-term memory in light of the current views on this matter, it seems as if the new model does not so much refute the current views, but rather, refines them. The new model does not argue that protein synthesis is unimportant to long-term memory storage, but instead says that it exclusively provides protein replenishment, while only post-synaptic modifications are capable of allowing long-lasting synaptic modifications.

Future Research:

As this recently proposed theory for long-term memory storage is still very new, more research is needed before this model can be generally accepted. Since this model requires continuous updating of neural networks, substances designed to inhibit post-translational modifications should be effective in disrupting memory days, months, or even years after the initial learning event takes place. Important experimental evidence for this new theory will be to show that interference can occur at long durations after initial learning event.

Why is This Controversy Important?

A more accurate description of long-term memory will help address issues of memory loss involved in mental retardation, aging, Alzheimer's Disease, etc. A better understanding of this area could also lead to the development of chemical agents that could potentially help prevent this loss.
References


