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Visions & Reflections

In search of molecular memory: experience-driven protein synthesis

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The formation and maintenance of memories is one of the most intriguing functions of the brain. The human brain has the remarkable ability to store and retrieve past experiences for years and in some instances decades. Of late, extensive effort has been put into reducing the process of learning and memory to the cellular and molecular level [see ref. 1]. The underlying logic is that if we can understand how an individual neuron remembers we might then extrapolate this function to groups of neurons residing in areas of the brain involved in memory formation and storage. One such area, residing in the medial temporal lobe, is called the hippocampus. This structure is especially important for explicit or declarative memory—the recall of information pertaining to people or places [2]. The process of protein synthesis is a critical event in the encoding of long-term (explicit) memory. Thus, a great deal of research emphasis has been placed on the molecular basis of this experience-driven protein synthesis in the hope that it will elucidate the foundations of memory.

The principal loci of cell-cell communication in the brain are synapses. Since synaptic transmission encodes information in the brain, the engram for memory may lie in the ability of the synapse to use stable modifications to remember its excitatory history. This form of synaptic plasticity was postulated in 1949 by Hebb [3], and was first described experimentally in the early 1970s as a long-lasting increase in synaptic strength following robust synaptic activity [4, 5]. The long-lasting potentiation (now called long-term potentiation or LTP) that Bliss and colleagues described in the hippocampus has become integral to our present theories for how memories are encoded [6, 7]. Although much of the following discussion centers on LTP, it is important to note that synaptic plasticity is a bidirectional process and that a synaptic weakening or long-term depression (LTD) is likely to play a role in memory as well [8, 9].

Protein synthesis and memory

One clue linking synaptic plasticity to memory formation is the common requirement for new protein synthesis [10–13]. This requirement was established in behavioral studies carried out primarily in the 1970s and early 1980s [reviewed in ref. 10]. In many of these studies, animals were injected with protein synthesis inhibitors just prior to training. The general finding was that protein synthesis-deficient animals would learn the given task at approximately the same rate as controls, but failed to retain their newly acquired knowledge. These results suggested that learning a task is mechanistically distinct from remembering it. In learning, short-term neuronal changes occur that are protein synthesis independent, whereas in memory formation, long-term neuronal changes require new protein synthesis.

Remarkably similar protein synthetic profiles are characteristic of some forms of synaptic plasticity. For example, the initial synaptic potentiation in the CA1 region of the hippocampus is protein synthesis independent but a long-lasting potentiation requires new translation. This dichotomy has been demonstrated for both

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synaptic potentiation (LTP) as well as synaptic depression (LTD) throughout the brain [12, 14].

Experience-driven protein synthesis is likely to occur via two mechanisms. The first is the production of new mRNAs via the activation of transcription factors within the nucleus. These newly synthesized mRNAs can potentially be translated in either the cell soma or the dendrite. The second mechanism is regulated protein synthesis occurring through the translational activation of mRNAs already located in the dendrite. Indeed, the interplay between protein products translated at the synapse and proteins generated as a result of transcriptional activation in the nucleus may form one basis of the ability of the neuron to remember (fig. 1).

Transcription regulation

There is substantial evidence from a wide range of systems that synaptic plasticity and long-lasting memory can be mediated by activation of the transcription factor, cAMP-responsive element binding protein (CREB) [see refs 15, 16]. In *Drosophila*, long-term memory is blocked when CREB function is disrupted by a dominant negative form of CREB [17]. In *Aplysia*, CREB-mediated protein synthesis is critical for the expression of long-term facilitation (LTF) at sensory neuron to motorneuron synapses [18]. In *Aplysia*, this LTF is required for learning in the form of sensitization (a strengthening of a reflex response to a previously neutral stimulus). In mammals, though CREB-mediated protein synthesis in the hippocampus is stimulated in mice during protocols that produce memory [19, 20], targeted disruption of the CREB gene yields varying degrees of memory impairment, suggesting that more complex pathways may compensate for the loss of CREB [21, 22]. Nonetheless, transcriptional activation seems likely to be one step in the process from synaptic activity to memory.

Dendritic translation regulation

One mechanism that has been proposed for both generating the signal from the synapse to the nucleus and for the stable insertion of new proteins at activated synapses is local translation of mRNA located in the synaptodendritic domain [23]. Local translation was first proposed following the identification of polyribosomes [24] and specific mRNAs [25] in the dendrites of hippocampal neurons. More recent studies have implicated dendritic protein synthesis in some forms of hippocampal LTP [26, 27] and metabotropic glutamate receptor-mediated LTD [28]. The molecular basis for the regulation of local translation is also now emerging

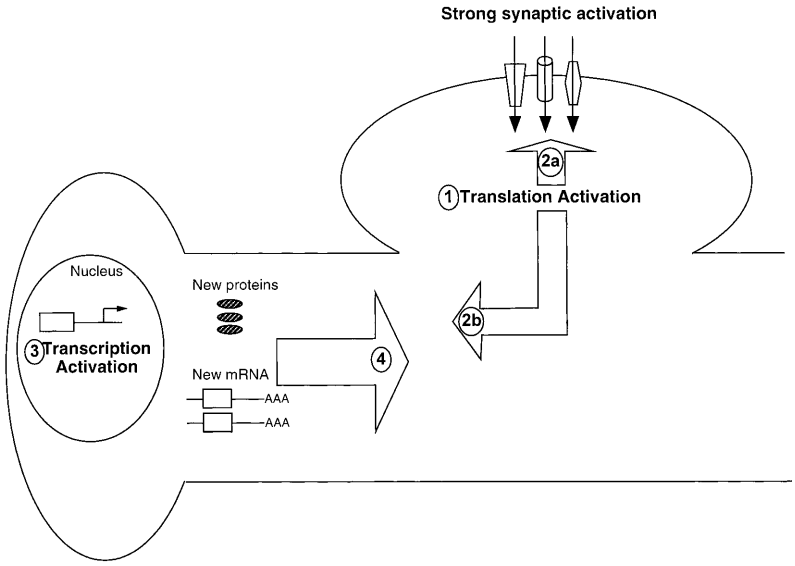

Figure 1. Model for experience-induced protein synthesis that might form the molecular basis of memory. Strong synaptic activation leads to current flow through both ligand-gated and voltage-dependent channels. Synaptic activation leads to the translational activation of dormant mRNA located at or near the synapse (1). Synaptic protein synthesis could directly affect synaptic signaling (2a) as well as generate a signal back to the nucleus (2b) which leads to transcriptional activation of new gene products (3). The newly synthesized mRNA and proteins are then transported back to the previously activated synapse where they are thought to stabilize and maintain synaptic changes (4).

[23, 29]. The general hypothesis is that mRNAs located in the dendrite are translationally dormant until synaptic activity drives them into a translationally active state. The protein products of this translation could act synaptically, or as has been demonstrated in *Aplysia*, could generate the signal which is then transmitted back to the cell nucleus [30, 31]. Regulation of synaptodendritic translation is dependent upon targeting, localization, and translational activation of specific mRNAs to the synapse. Due to space restrictions, we will address only the process of translational activation, though potential mechanisms for targeting and localization are also under intense investigation [29, 32–36].

Recently, a mechanism has been described such that dormant mRNA can be activated, in an experience-dependent fashion, through a process known as cytoplasmic polyadenylation [37]. Here, a mRNA binding protein called cytoplasmic polyadenylation element-binding protein (CPEB) is localized to synapses on hippocampal neurons. CPEB was first discovered in *Xenopus* oocytes, where it regulates translation of mRNAs that contain a specific binding element (CPE) in the 3′ untranslated region. During oocyte maturation, CPEB initially inhibits translation during a dormant stage [38, 39]; however, following stimulation, CPEB induces cytoplasmic polyadenylation and the initiation of translation [40].

We have shown that a CPE-containing mRNA is polyadenylated in the rodent brain in an experience-dependent fashion [37]. The mRNA we examined in that study encodes the alpha subunit of calcium/calmodulin-dependent protein kinase II (α-CaMKII), a protein implicated in processing memory [41]. Interestingly, the polyadenylation of α-CaMKII mRNA was also associated with an increase in the amount of α-CaMKII protein in the synaptic fraction of the visual cortex, a rise that is blocked by the translation inhibitor cycloheximide. Together, these findings suggest that translation of α-CaMKII was triggered by activity and regulated by polyadenylation. In addition, CPE-mediated protein synthesis in cultured hippocampal neurons is regulated by NMDA receptor activation [42]. It is important to note that there are undoubtedly other mechanisms of translational regulation in the brain [43]. For example, the fragile X mental retardation protein is thought to be synthesized in the synaptodendritic region following the activation of the metabotropic glutamate receptor [44]. This process is thought to involve the activation of protein kinase C and the subsequent activation of another kinase, p90rsk, that then translocates to polyribosomes and stimulates translation [45]. Elucidating the mechanisms of translational activation at the synapse and determining their stimulus specificity will be an important step in deciphering the memory code.

Roles for synaptic protein synthesis

In addition to synthesizing proteins that directly impact synaptic efficacy (e.g., α-CaMKII), how else could local protein synthesis direct long-term cellular changes? Following 'appropriate' synaptic activation, calcium influx is thought to trigger the production of a signal that can induce nuclear transcription [46] (fig. 1). The nature of the synapse-to-nucleus signal is still unknown, however, protein kinase A and calmodulin have effects at both the synapse and the nucleus, making them candidates for the signaling molecule [47, 48]. In *Aplysia*, where this process is more readily studied, this signal is not generated if a translation inhibitor is present at the activated synapse, suggesting that the signaling molecule is produced by local translation [30]. Furthermore, in the absence of local (synaptic) protein synthesis, synapses are less capable of maintaining both LTF and the accompanying synaptic growth [31]. This suggests that activity-driven local protein synthesis can have long-lasting effects on synaptic efficacy and structure, perhaps stabilizing these changes.

Molecular memory: the long haul

An apparently daunting obstacle to molecular memory is the inherent fragility of the molecules themselves and their persistent turnover. Any theory proposed for molecular memory must account for the regeneration of the molecules involved [1, 49, 50]. Feedback loops involving protein synthetic pathways either in the cell body or the dendrite are therefore attractive models for memory storage (fig. 1). We feel that a feedback loop involving an interplay between synaptic protein synthesis and transcriptional activation of new gene products could lead to the kind of stable changes required for long-term plasticity. Critical to deciphering this type of memory loop will be the identification of the proteins involved. Although mediators of CREB activity have been described [48, 51–53], the putative synapse-nucleus signaling molecule has not (fig. 2). Because many signaling molecules have distinct roles both at the synapse and the nucleus, synaptic translation could potentially produce two pools of these molecules. One pool would act remotely (signal to nucleus) while the other would be utilized locally to modify or activate signaling cascades at the synapse (fig. 2). The production of new mRNA and protein products from the nucleus would then act to reinforce and resupply these signaling molecules at the synapse, thus ensuring a long-lasting and stable change.

The formation of sustainable memory is undoubtedly an extremely complicated process that requires an interplay between many different mechanisms. Some of the molecular mechanisms involved are now being de-

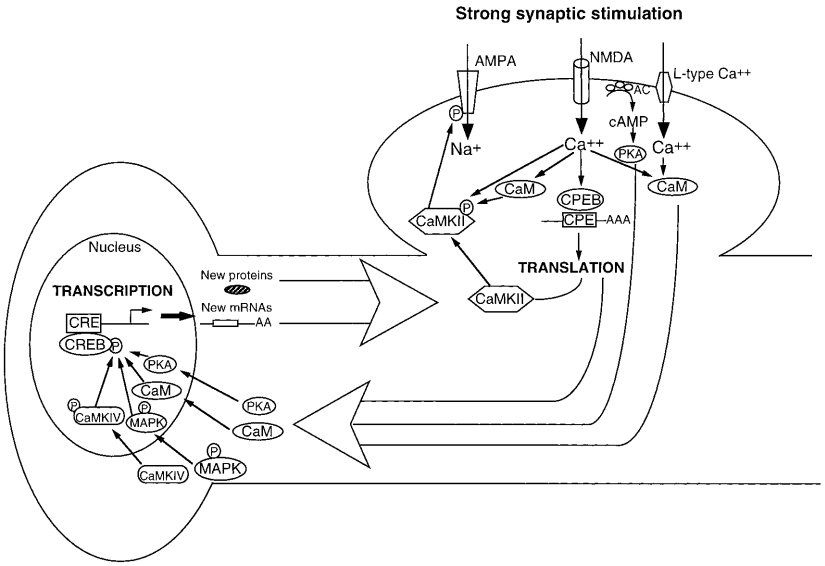

Figure 2. Molecular mediators of synaptic plasticity in a central nervous system neuron that might form the molecular basis of memory. Strong synaptic activation leads to current flow through both ligand-gated channels (e.g., glutamatergic AMPA and NMDA receptors) and voltage-dependent channels (e.g., L-type Ca^{2+} channels). Calcium entry through both the NMDAR and L-type channel result in an increase in the levels of calmodulin (CaM) and elevation of cAMP and protein kinase A (PKA) activity. NMDAR activation could also lead to the translation of CPE-containing mRNAs (see text) that directly affect synaptic signaling (α-CaMKII). Translation by this or other methods could also generate a signal that will lead to the phosphorylation and activation of nuclear CREB. The resultant gene products are then targeted to the dendrites where they are utilized by activity-tagged synapses.

scribed yet many of the specifics of the signaling process are still a mystery. Identification of experience-inducible proteins, how they are sustained, and their physiological consequence is now on the horizon. Ultimately, understanding these processes may help us decipher the memory code and enable us to enhance the ability to store and retrieve memories.

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