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Mechanisms of Translation Control Underlying Long-lasting Synaptic Plasticity and the Consolidation of Long-term Memory

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Abstract

The complexity of memory formation and its persistence is a phenomenon that has been studied intensely for centuries. Memory exists in many forms and is stored in various brain regions. Generally speaking, memories are reorganized into broadly distributed cortical networks over time through systems level consolidation. At the cellular level, storage of information is believed to initially occur via altered synaptic strength by processes such as long-term potentiation (LTP). New protein synthesis is required for long-lasting synaptic plasticity as well as for the formation of long-term memory. The mammalian target of rapamycin complex 1 (mTORC1) is a critical regulator of cap-dependent protein synthesis and is required for numerous forms of long-lasting synaptic plasticity and long-term memory. As such, the study of mTORC1 and protein factors that control translation initiation and elongation have enhanced our understanding of how the process of protein synthesis is regulated during memory formation. Herein we will discuss the molecular mechanisms that regulate protein synthesis as well as pharmacological and genetic manipulations that demonstrate the requirement for proper translational control in long-lasting synaptic plasticity and long-term memory formation.

Keywords

memory; consolidation; long-term potentiation; protein synthesis; mTOR; 4E-BP; eIF4E; S6K1; eIF2α

I. The Study and Characterization of Memory Systems

A. Historical Overview

The term consolidation refers to the progressive post-acquisition stabilization of long-term memory and to the phase(s) of the memory stabilization process[1,2]. During the consolidation process, newly acquired information is gradually transformed into long-term memory. Historically, the term consolidation is attributed to Müller and Pilzecker, who first studied the acquisition and retrieval of syllable pairs in human subjects[1,2]. These studies demonstrated that the correct recall of syllable pairs required some time before reaching a certain success criterion, and that the presentation of new material before the consolidation of syllable pairs damaged its recall. Thus, new memories require time to be stabilized or “consolidated”. Furthermore, these studies indicated that there is a post-training time window during which it is possible to interfere with newly learned information. As a result, new information is susceptible to destruction before being consolidated into a memory. Overall, these studies pointed out two important properties of the consolidation process:

time dependency and distinct stages of memory. Indeed, during the consolidation process, memory is converted from a labile to stable form in a time-dependent manner that eventually becomes resilient to interference.

The idea that memory is gradually transformed was hypothesized first over a hundred years ago. In 1882, the French psychologist, Théodule-Armand Ribot, proposed that memories are gradually reorganized with the passage of time[3]. Moreover, he made the observation that following brain injury the degree of memory loss was correlated with the age of the memory. Specifically, there was a greater loss of newer memories, compared to older memories. This time-dependent component of memory reorganization is known as Ribot's Law and provides the basis for research performed on temporally-graded retrograde amnesia. Today, we know of this memory transformation process as systems consolidation.

One of the most well described cases of a patient that exhibited retrograde amnesia was patient Henry Gustav Molaison (known for many years as patient H.M. until after his death in 2008, when his name was finally revealed) first described by Milner and Scoville[4,5]. H.M. was affected by severe temporal lobe epilepsy, which was treated by the surgical transection of his hippocampus, parahippocampal gyrus, and amygdala. Following this procedure, H.M. suffered from moderate retrograde amnesia; however, the amnesia was not complete. Indeed, only his most recent memories were lost, whereas memories from his childhood were retained.

H.M. also suffered from persistent anterograde amnesia, which is the inability to form new memories, specifically restricted to declarative memory, which is defined as memory that can be recollected consciously. His impairment included the inability to form new episodic (memory of events) and semantic (memory of general knowledge or facts) information. However, other forms of non-declarative memory, such as procedural memory and perceptual learning, were largely spared. Overall, the studies of H.M.'s memory deficits indicated that memory is not a unitary phenomenon, but rather involves multiple memory systems that exist simultaneously in different brain regions. In fact, lesions in the hippocampus critically impaired declarative memory but left procedural memory intact, suggesting that the latter is different from the former and is not reliant on the hippocampus. Moreover, these lesions only impaired recent memories, indicating that hippocampus is the anatomical locus where newly acquired memories reside, whereas older memories are stored permanently elsewhere.

It is clear that the general term "consolidation" includes at least two different types of processes characterized by distinct temporal dynamics (Figure 1). Heretofore we refer to fast consolidation processes as synaptic consolidation, which is completed in hours after training and involves the stabilization of changes in synaptic connectivity within localized circuits. By contrast, systems consolidation is a more prolonged process and requires the gradual reorganization of brain regions that support memory. Hereafter we will focus on the molecular mechanisms underlying synaptic consolidation.

B. Cellular Mechanisms of Memory Consolidation

Memory is encoded and stored in the brain via carefully regulated interactions between neuronal networks. Synapses are the essential unit of memory and are the site of electrochemical communication between neurons. These connections are “plastic”, in that the physiological responsiveness (i.e., the “strength” of the synaptic connection) is modifiable. Importantly, changes in synaptic strength (synaptic plasticity) also are defined temporally, with some alterations lasting only seconds and others persisting over the lifetime of the organism[6,7]. The most well established model for persistent activity-dependent strengthening of individual synapses is long-term potentiation (LTP).

The first full description of LTP by Bliss and Lomo[8] reported that trains of high-frequency stimulation (HFS) to the perforant path caused a sustained increase in efficiency of synaptic transmission in the granule cells of the dentate gyrus in the hippocampus of the rabbit. These findings suggested that the synaptic changes underlying certain forms of learning and memory might be similar to the mechanisms responsible for LTP. Indeed, activity-dependent synaptic plasticity at appropriate synapses during memory formation is necessary and sufficient for storage of information. Some of the characteristics of LTP, such as cooperativity, associativity, input specificity[9], and durability[10], have been put forth as solid arguments in support of the hypothesis that LTP is a biological substrate for at least some forms of memory.

Other similarities have been described between memory consolidation and synaptic plasticity. For instance, there are common molecular changes that occur after the induction of LTP and during memory acquisition, which can be targeted and disrupted using the same pharmacological agents. For example, memory is divided into two different temporal phases: short-term memory, which lasts for a few hours, and long-term memory, which persists for much longer. At the cellular level, the storage of long-term memory is associated with changes in gene expression, *de novo* protein synthesis, and the formation of new synaptic connections[11–13]. Consistent with these observations, protein synthesis inhibitors block long-term memory, but leave short-term memory unaffected, suggesting that stable long-lasting memories rely on new gene expression triggered at the time of the experience[14–18]. Similarly, LTP consists of distinct phases involving different molecular mechanisms. The early phase of LTP (E-LTP), which lasts 2–3 hours, is independent of protein synthesis, whereas more long-lasting LTP (L-LTP), which persists several hours *in vitro* and either weeks or months *in vivo*, requires the synthesis of new proteins[9]. Thus, memory and synaptic plasticity share similar properties and require similar molecular events for their persistence.

Hippocampal circuits have been a valuable system for studying synaptic plasticity in the context of information storage mechanisms in the brain. Typically, synaptic plasticity is measured using electrophysiological techniques. Moreover, the hippocampus is one of the principal brain regions studied because of the presence of the trisynaptic circuit (see below). The laminar anatomical arrangement of the hippocampus allows for the preservation of this circuit in transverse slices, which facilitates experimentation. Additionally, the neuronal pathways within the hippocampus display remarkably pronounced plasticity[19].

The trisynaptic circuit refers to the major hippocampal synapses and is comprised of three main groups of excitatory connections: 1) the mossy fiber pathway generated by axons of granule neurons of the dentate gyrus that synapse onto CA3 pyramidal neurons, 2) the Schaffer collateral pathway consisting of axonal projections of the CA3 pyramidal neurons that synapse onto CA1 pyramidal neurons; 3) the efferent fibers originating in CA1 that project to sub-cortical and cortical neurons. Most of the electrophysiological experiments described in this chapter were performed on the synaptic connections between CA3 and CA1 pyramidal neurons[20].

The standard recording arrangement used to study synaptic plasticity between CA3 And CA1 pyramidal neurons is achieved by placing a stimulating electrode proximal to the CA3 Schaffer collateral axons. The recording electrode is placed into the stratum radiatum of the CA1, where it will record electrical activity induced by the stimulation of axonal inputs originating in the CA3 (Figure 2A). LTP can be induced using a wide variety of induction protocols at these synapses. The standard protocols include a either single (for E-LTP) or repeated (usually 2 to 4 trains for L-LTP) delivery of one-second 100 Hz stimulation (HFS), where repeated trains of 100 Hz are separated by 20 seconds or greater (Figure 2B). A variation of this protocol employed to induce LTP is the use of theta burst stimulation (TBS). In this protocol, 3 trains of 100 Hz are delivered with and inter-train intervals of 20 seconds[19].

The dependence of protein synthesis for long-lasting memory correlates with the necessity of *de novo* protein synthesis for synaptic plasticity[11]. The classical view of how translation impacts L-LTP is that proteins are synthesized in the cell body and delivered to the potentiated synapses. However, it is now clear that proteins can be synthesized locally in dendrites in response to changes in synaptic plasticity. For instance, local dendritic protein synthesis has been demonstrated with biochemical fractionation techniques, which permits the isolation of dendritic fragments (e.g. synaptoneurosome preparations). In these studies, different kinds of stimulation, such as membrane depolarization with high K^+[21], brain-derived neurotrophic factor (BDNF)[22], and group I metabotropic glutamate receptor agonists (mGluRs)[23], were shown to increase protein synthesis. Several proteins were shown to be synthesized, including the α subunit of calcium/calmodulin dependent protein kinase II (α-CaMKII), Arc, and fragile X mental retardation protein (FMRP), respectively. Overall, these experiments are consistent with the idea that *de novo* protein synthesis occurs in active synapses, which supports the notion that local protein synthesis is involved in synaptic plasticity.

The demonstration that local protein synthesis occurs in synaptoneurosome preparations was confirmed with studies in neuronal cultures and brain slices. For example, it was shown that in hippocampal slices BDNF-induced synaptic plasticity is blocked by protein synthesis inhibitors[24]. In the same study, Schaffer collateral-CA1 synapses isolated from their pre- and post-synaptic cell bodies still exhibited protein synthesis-dependent plasticity[24]. A similar dependence on *de novo* protein synthesis has also been observed for a different form of synaptic plasticity: metabotropic glutamate receptor-dependent long-term depression (mGluR-LTD) at the same synaptic location[25,26]. Moreover, in cultured hippocampal neurons, the presence of local protein synthesis was demonstrated first with a protein

synthesis reporter[27]. In this study, the reporter contained the coding sequence of green fluorescent protein (GFP), which was flanked by the 5′ and 3′ untranslated regions of α-CaMKII, which confers mRNA localization and translational regulation. After stimulation with BDNF in isolated dendrites, the increase in GFP fluorescence was followed with time-lapse imaging[27]. These experiments strongly suggest that BDNF-induced synaptic plasticity requires local translation machinery and thus, dendritic protein synthesis.

C. Behavioral Characterization of the Memory Trace

The study of molecular and synaptic neuronal modifications triggered by learning, which enables long-term storage of memories, is accompanied by separate studies that have been performed to investigate the functional role of these modifications in behavioral paradigms. These studies have contributed to the characterization of molecular mechanisms in memory consolidation.

As discussed earlier, a distinct feature of long-term memories is that it is more stable than short-term memory and thus more resistant to interference. Experimentally, this window of susceptibility depends on the behavioral task and the type of interference or blockers used, and can range from minutes (e.g. electroconvulsive shock, ECS) to hours (e.g. distractor tasks, protein synthesis drug inhibitors).

Initially, ECS was one of the first blockers used to interfere with the formation of long-term memory, and was shown to mimic retrograde amnesia in animal models[11]. In these studies, rodents showed impaired memory retention only when ECS was given shortly after training, but had no effect when administered after a longer time period following training [28,29]. Although the ability of ECS to disrupt memory is restricted to minutes after memory formation, certain pharmacological agents are capable of interfering with memory more robustly. Furthermore, these pharmacological treatments can result in effects that are more persistent, such as the case with protein synthesis inhibitors. The first demonstration that protein synthesis is required for the formation of long-lasting memories was provided by the work of Louis and Josefa Flexner. They demonstrated that temporal lobe injections of the protein synthesis inhibitor puromycin after learning were effective in blocking long-term memory in mice[30].

Later, a series of pharmacological experiments in learning and memory paradigms confirmed that protein synthesis occurs after training and is required for the formation of long-term memory. Importantly, blocking protein synthesis does not impair the acquisition of short-term memory (extensively reviewed in [11]). Commonly used protein synthesis inhibitors in memory studies include: puromycin, anisomycin, acetoxycycloheximide, and cycloheximide. In a typical pharmacological study, an animal is treated with the protein synthesis inhibitor shortly after training. Following this, acquisition of short-term memory and long-term memory formation are tested[31]. The expected outcomes from these studies are that the acquisition of short-term memory will be intact, whereas the formation of long-term memory will be impaired. Thus, consolidation of short-term memories into long-term memories is dependent on protein synthesis[32,33].

II. Mechanisms of mTOR-dependent Translational Control

The mammalian target of rapamycin (mTOR) is an evolutionarily conserved kinase that has emerged as one of the critical checkpoints of protein synthesis and plays a major role in synaptic plasticity and memory. mTOR activity is modulated by a wide variety of signals, ranging from neurotransmitters to changes in energy metabolism status, which result in the tight control of cap-dependent protein synthesis through multiple downstream effectors.

A. Upstream Regulation of mTOR

mTOR is activated by multiple neurotransmitters and neurotrophic factors via neuronal surface receptors and channels. For instance, a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA-R), N-methyl-D-aspartate receptors (NMDA-R), dopamine receptors (DRs), metabotropic glutamate receptors (mGluRs) and BDNF receptors (i.e. TrkB-R) activate mTOR and are vital for the induction and expression of synaptic plasticity and memory[34]. mTOR acts as a node of convergence downstream of these receptors as well as several signaling pathways that include phosphoinositide-dependent kinase-1 (PDK1), phosphatidylinositol 3-kinase (PI3K), PKB/Akt, and tuberous sclerosis complex proteins 1 and 2 (TSC1/2)[34–36]. The extracellular signal-regulated kinase (ERK) signaling pathway also activates mTOR, although it is unclear whether this results from phosphorylation of TSC1/2 by ERK or via its downstream effector p70 S6 kinase 1(S6K1)[37,38].

In addition to activation via neurotransmitters, mTOR signaling is also regulated by amino acid and cellular energy status. Amino acid starvation typically inhibits mTOR signaling via a mechanism that involves the PI3K-related protein Vps34 and is independent of TSC1/2-Rheb. On the other hand, reduction of adenosine triphosphate (ATP) levels inhibits mTOR signaling via AMP-activated protein kinase (AMPK), which is a sensor of cellular energy status, and leads to the subsequent phosphorylation of TSC1/2[39,40]. All of these signals are ultimately funneled and integrated in the control of cap-dependent protein synthesis through direct or indirect regulation of mTOR downstream effectors.

mTOR is typically the catalytic component in one of two multi-protein signaling complexes, termed mTOR complex 1 and 2 (mTORC1 and mTORC2, respectively)[41]. (Figure 3) The other proteins that are components of the mTORCs control subcellular localization, substrate specificity, and signaling. Because functional mTOR is always observed in one of the two complexes, hereafter we will refer to only mTORCs.

mTOR is in mTORC1 when it is associated to the regulatory scaffolding protein Raptor. The function of Raptor is to bind proteins containing a short amino acid sequence called the mTOR signaling (TOS) motif and shuttle them to the mTOR catalytic subunit. The best-characterized TOS motif-containing proteins are those that are involved in the regulation of protein synthesis, S6K1 and eIF4E-binding protein (4E-BP)[34,42]. mTORC1 is sensitive to inhibition by the antifungal rapamycin because of the competition between Raptor and the rapamycin-FKBP12 complex for binding to the FRB domain[43,44]. In addition to Raptor, mTORC1 contains other proteins that fall into two categories: common constituents of the mTORCs, such as the G beta-like protein family member LST8, and exclusive constituents

of mTORC1. For instance, proline-rich Akt/PKB substrate of 40 KDa (PRAS40) is only present in mTORC1. PRAS40 is important for the regulation of mTOR-Raptor interactions and mTOR signaling[45,46]. The best-characterized function of mTORC1 during synaptic plasticity and memory is the regulation of protein synthesis, achieved by controlling two critical downstream effectors proteins: S6K1 and 4E-BP, which are involved in the initiation and elongation phases of translation (see below).

The other mTOR complex, mTORC2, is formed when mTOR is associated with the rapamycin-insensitive companion of mTOR, Rictor. Although insensitive to acute rapamycin exposure, prolonged chronic treatment also disrupts mTORC2 function[44,47]. mTORC2 contains its own unique component, the SAPK-interacting protein1 (Sin1), which is believed to be required for the proper formation of mTORC2, as well as for its activity[48–50]. Based on its unique composition, mTORC2 acts on a different set of targets and regulates different intracellular processes. There is less known about the role of mTORC2 in synaptic plasticity and memory, but recent studies with mice that lack Rictor have demonstrated that similar to mTORC1, mTORC2 is required for L-LTP and long-term memory[51].

The process of protein synthesis results in the sequential translation of mRNA into amino acids that are added to a growing polypeptide chain to make a protein. The biochemical signaling mechanisms that regulate protein synthesis can be divided in three consecutive phases: initiation, elongation, and termination. These phases are facilitated and regulated by proteins termed eukaryotic initiation factors (eIF), elongation factors (eEF), and release factors (eRF) based on the particular phase in which they are active. mTORC1 controls a number of components involved in the initiation and elongation phases of translation.

It is important to note that mTORC1 signaling likely contributes both to the short-term (minutes) activation of translation and to long-term (hours) increases in the translational capacity of neurons by regulating the synthesis of ribosomal proteins and other translational components. Both forms of translational regulation are important for synaptic plasticity and memory formation.

B. Translation Initiation

The initiation phase of protein synthesis refers to the assembly of the small ribosome subunit (40S) to mRNA (mediated by eIF4 group of eIFs) and the recruitment of the initiator methionyl-tRNA (Met-$tRNA_i^{Met}$) to the first AUG start codon. The recognition of the first AUG codon at the beginning of the coding region of the mRNA occurs after a process termed “scanning”, where the 5′-untranslated region (5′-UTR) of the mRNA is inspected for a suitable start codon[34,35,52,53].

A wide variety of signals are transduced and integrated to regulate the initiation phase of protein synthesis. This phase is controlled by two main mechanisms: phosphorylation of eIFs, which modulates their activity and the association of regulatory proteins to specific sequences of mRNAs, usually in either the 5′- or 3′-untranslated regions (UTRs), which either promotes or represses their translation. In the next sections we will mostly focus on the molecular mechanisms modulating the initiation phase of protein synthesis. We will also review the regulatory mechanisms acting at the 5′ end of the mRNAs[52–54]. Additional

proteins also have been described that regulate translation by acting at the 3′ end of mRNAs, such as cytoplasmic polyadenylation element binding proteins (CPEB) and polyA-binding proteins (PABP), which will not be discussed in this chapter[55–59].

The recruitment of the Met-tRNA$_i^{Met}$ to the 40S ribosomal subunit starts with the binding of the translation factor eIF2 to the initiator Met-tRNA$_i^{Met}$ in a GTP-dependent manner. This complex associates to the small (40S) ribosomal subunit together with other initiation factors, such as eIF1, eIF1A, eIF3, and eIF5 to form a 43S pre-initiation complex. The association of the 43S pre-initiation complex to the mRNA is facilitated by the eIF4 group of eIFs and structures present on the mRNA. The structures important for translation initiation are the 7-methyl-GTP (m^{7}GTP) cap at the 5′ end and the poly(A) tail at the 3′ end of the mRNA. The 5′ and 3′ structures are bound by specific proteins, such as the cap-binding protein eIF4E and PABPs, respectively[34,35,52,53,60,61].

The eIF4 group of eIFs involved in the initiation of translation form the cap-binding complex, eIF4F, which consists of eIF4E, the DEAD-box RNA helicase eIF4A, and the scaffolding protein eIF4G. eIF4G binds eIF3 of the 43S complex and circularizes the mRNA by interacting simultaneously with eIF4E and PABPs (Figure 4). The circularized mRNA enhances the association of the cap-binding complex eIF4F and, consequently the 48S complex formation. The eIF4A helicase unwinds the secondary structure at the 5′ end of the mRNA, thus facilitating ribosomal binding and scanning. The helicase activity of eIF4A is enhanced by eIF4B when it is phosphorylated by S6K1, which in turn is controlled by mTORC1 (see below)[34,35,44,52–54,60,61].

The formation of the cap-binding complex eIF4F is thought to promote the binding of the 43S complex to the mRNA, which forms the 48S pre-initiation complex. This complex scans the mRNA from the 5′ end and stops in proximity of the first AUG start codon. Recognition of the start codon triggers the hydrolysis of GTP by eIF2 and promotes the release of the eIFs from the 48S complex. This allows the complex eIF5B, another GTP-bound protein, and the large ribosomal subunit (60S) to join the complex and form the 80S ribosome. Then, eIF2-GDP is released from the 48S complex and the guanine exchange factor (GEF) eIF2B catalyses the exchange of GDP for GTP on eIF2 , thereby recycling eIF2 for another round of translation[34,35,52–54].

C. Translation Elongation

The elongation phase of protein synthesis refers to the translocation of the ribosome along the mRNA, the decoding of the mRNA codons into amino acids, and the formation of new peptide bonds that extend the newly synthesized polypeptide chain. This phase of protein synthesis requires two factors, eEF1 and eEF2, and represents the second checkpoint of the regulation of translation[62,63].

eEF2 is a GTP-binding protein that promotes the translocation step of elongation. During this phase, the ribosome moves along the mRNA and the peptidyl transfer RNA (tRNA) translocates from the A site into the P site on the ribosome, following formation of the new peptide bond. eEF2 undergoes phosphorylation at Thr56 in its GTP-binding domain which inhibits the binding of eEF2 to ribosomes, thereby reducing its activity. The phosphorylation

of eEF2 is catalyzed by a highly specific Ca^{2+}/calmodulin-dependent kinase, eEF2 kinase[63–65].

Stimuli which induce protein synthesis leads to the rapid dephosphorylation of eEF2, which results in increased activity and accelerated elongation[66]. The dephosphorylation of eEF2 appears to be caused by inhibition of eEF2 kinase. Both of these events are blocked by rapamycin[66] suggesting that mTORC1/S6K1 is involved in the control of eEF2 function. The activity of eEF2 kinase is modulated via phosphorylation at several sites. The residue Ser366 is phosphorylated by S6K1 and RSK, leading to the inhibition of eEF2 kinase activity, at least at submaximal Ca^{2+} ion concentrations[67]. Phosphorylation at Ser78 is controlled by mTORC1 and results in the inhibition of the association calmodulin/eEF2 kinase, thus blocking its activity[68]. Also, phosphorylation at Ser359 is regulated by mTORC1 and inhibits eEF2 kinase activity[69]. Thus, mTORC1 activates translation elongation either directly or via S6K1. The mTORC1-dependent regulation of translation at the elongation phase is an important mechanism that, in conjuction with the regulation at the initiation phase, tightly controls the set of synthesized proteins under certain stimuli[68,69].

III. Translational Control Molecules Involved in Synaptic Plasticity and Memory

Genetic engineering of mice is an approach that has been utilized to selectively and subtly manipulate discreet molecular effectors that regulate protein synthesis. The study of a number of mutant mice that either lack or overexpress translational control molecules has resulted in the accumulation of a large body of evidence supporting a role of protein synthesis in memory formation. The role several of the aforementioned translational control molecules in memory formation have been validated using a variety of commonly used behavioral tests, which are described in Box 1.

Box 1

Behavioral Assessment of Learning and Memory

Morris Water Maze (MWM)

The MWM is a commonly used task that measures hippocampus-dependent memory by training rodents to acquire the location of a hidden platform within a circular pool of water. Memory is measured in several ways, the first being the determination of the latency to locate the hidden platform. After a given amount of training, rodents will acquire the platform position and the latency to find the platform will decrease. Memory also is measured using a probe test. In this phase of the task, the platform is removed and memory is measured both by the number of times the rodent crosses the platform position and by the amount of times the rodent spends in the quadrant in which the platform was formerly located, also known as the target quadrant. One of the most critical aspects of this test is the presence of visual cues surrounding the water maze such that the rodents can use these cues to guide them in learning the platform position.

Fear Conditioning (FC)

Auditory fear conditioning is behavioral test that measures a type of associative memory. In this task a conditioned stimulus (CS), typically a tone, is paired with an unconditioned stimulus (US), typically a footshock. Two types of memory can be tested following this CS-US pairing: contextual fear memory and cued fear memory. To measure contextual fear memory, rodents are placed in the context in which they received the CS-US pairing and memory is measured by the time spent freezing in this context. Cued fear memory, on the other hand, is measured by the amount of time spent freezing during the CS presentation in a novel context. Importantly, contextual fear memory is hippocampus-dependent, whereas cued fear memory is not.

Condition Taste Aversion (CTA)

In the CTA task, rodents are introduced to a novel taste and immediately injected with a malaise-inducing agent such as LiCl. Memory of this association is measured using a taste aversion index, where a higher value represents a greater memory of the association. Latent inhibition of CTA is a similar task, but in this task rodents are pre-exposed to the novel taste, which reduces the aversion index in the memory phase of the task.

Novel Object Recognition (NOR)

The novel object recognition task is a measure of the innate tendency of rodents to explore novel objects. Although the details vary from laboratory to laboratory, the basic framework of this task is the same. In the first phase of the task, the rodent is habituated to the testing arena, which does not contain any objects. This is designed to reduce any possible anxiety that may be associated with being placed in an unfamiliar environment that could confound behavioral outcomes. Following a set intertrial interval, the rodent is once again placed in the testing arena where the presence of two identical objects has been added, which they are allowed to explore. The last phase of the task occurs after another set intertrial interval and the rodents are placed back into the same environment; however, one of the familiar objects is replaced with a novel object. Because rodents display a tendency to explore novelty, they will spend a greater amount of time interacting with the novel object compared to the familiar object. Typically, this value is represented as a ratio of time spent interacting with the novel object, over the total time spend interacting with either object. A preference index of 0.5 would represent an equal amount of time spend with both objects; thus, a preference index > 0.5 reflects a preference for the novel object.

A. mTOR

1. mTOR Structure and Function—mTOR is a large (approximately 250 KDa) multi-domain serine/threonine kinase and member of the family of phosphoinositide (PI) 3-kinase-related kinases. mTOR is highly conserved from yeast to humans[34,70]. In mTORC1, it is a target of rapamycin, which is an immunosuppressant drug (see below). Structurally, mTOR is characterized by more than 20 HEAT (**H**untingtin, **E**longation Factor 3, **A** subunit of PP2A, **T**OR1) repeats located in at its N-terminal. The HEAT repeats fold into a helical structure that functionally interacts with regulatory proteins such as Raptor and Rictor[34,41,71,72].

The C-terminal portion contains FRAP and FATC domains required for the catalytic functions of mTOR. Adjacent to these domains is the FKBP12-rapamycin binding (FRB) domain that serves as binding site for the immunophilin FKBP12, which appears to occur only in the presence of rapamycin. The binding of the FKBP12-rapamycin complex to FRB disrupts the interactions of mTOR with Raptor, thereby inhibiting mTORC1. Other proteins interacting with mTOR, such as the Ras homolog enriched in the brain (Rheb), also binds the FRB domain[34,41,42,71–73].

The kinase catalytic domain (KIN) of mTOR is comprised of a region termed negative regulatory domain (NRD). NRD contains three amino acid residues (Thr2246, Ser2448, and Ser2481) that upon phosphorylation regulate mTOR kinase activity. Thr2246 is phosphorylated by AMPK and S6K1, Ser2448 is the target of Akt and S6K1, and finally, Ser2481 is an autocatalytic phosphorylation site. Phosphorylation of these residues, in particular, Ser2448, have been shown to be correlated with high mTOR kinase activity. Overall, the phosphorylation state of mTOR at these multiple sites represents an important mechanism of feedback regulation as some of them are substrates of downstream effectors of mTOR (i.e. S6K1)[44,68,72,74–76].

Deletion of the *mTOR* gene results in a lethal phenotype, suggesting that *mTOR* is an essential gene. Indeed, mice lacking *mTOR* die *in utero* shortly after implantation and embryonic development appears to be arrested at E5.5 with multiple developmental aberrations[77,78]. Consequently, the only method of studying the function of mTORC1 has been via its pharmacological inhibition. Rapamycin has been used widely to study mTORC1 signaling in long-lasting synaptic plasticity and memory formation across multiple experimental preparations and species.

2. The Role of mTOR in Synaptic Plasticity and Memory Consolidation—

Rapamycin (or sirolimus) is a macrolide produced by the bacterium *Streptomyces hygroscopicus*. It was named after the island of Rapa Nui where it was discovered first in a soil sample[79]. It has immunosuppressive and anti-proliferative properties, and is an FDA-approved immunosuppressant drug used clinically to prevent rejection after organ transplant.

Rapamycin is a potent inhibitor of mTORC1, which is defined as the complex of mTOR and the adaptor protein Raptor (see above). Rapamycin acts by binding FKBP12, the intracellular receptor of rapamycin, and disrupts the interaction between Raptor and mTOR, thereby blocking mTORC1 signaling[34,42,73,80].

The first demonstration of the involvement of mTORC1 in synaptic plasticity comes from experiments performed in rodent hippocampal slices where pretreatment with rapamycin abolished the late phase of L-LTP[36,81]. Moreover, L-LTP was sensitive to rapamycin only when the drug was bath applied during induction and not after the establishment of this form of synaptic plasticity[82]. These experiments suggest that activation of mTORC1 is required for the maintenance of L-LTP and that this molecular event is tightly regulated within a specific temporal window.

mTOR, its effector protein 4E-BP, and eIF4E are co-localized with postsynaptic markers, suggesting that rapamycin blocks L-LTP by inhibiting the postsynaptic translational

machinery[36,81]. Subsequently, it was shown that L-LTP leads to activation of mTORC1 signaling resulting in the translation of mRNAs, such as the one encoding αCaMKII[83,84] and eEF1A[85]. The eEF1A mRNA contains a 5′-UTR oligopyrimidine tract (TOP) sequence, which is one of the structures that operate as a regulatory element for protein synthesis. TOP mRNAs generally encode for ribosomal proteins and translation factors, and their translation is specifically regulated by mTORC1. Thus, mTORC1 controls the synthesis of proteins important for synaptic plasticity (i.e. αCaMKII) and amplifies translation by increasing the availability of factors involved in the protein synthesis machinery.

Consistent with the involvement of mTORC1 in synaptic plasticity, there is a great deal of experimental evidence that demonstrates the requirement of mTORC1-dependent protein synthesis in memory formation *in vivo*. For example, hippocampus-dependent long-term spatial memory has been shown to be mTORC1-dependent. To study the role of mTORC1 in the Morris water maze (MWM), rats were given post-training infusions of rapamycin in the dorsal hippocampus. Inhibition of mTORC1 with rapamycin blocked long-term memory in a retention test 48 hours after training[86]. Finally, consolidation of fear memory, an amygdala-dependent and fear-motivated learning task, also has been shown to be an mTORC1-dependent process. Rats trained in a cued fear conditioning paradigm exhibited increased phosphorylation of S6K1 and post-training infusions of rapamycin into the amygdala prevented the formation of cued fear memory[87].

Overall, pharmacological studies using rapamycin to inhibit mTORC1 signaling have shown that blocking this molecular signaling impairs the formation of long-lasting memories. However, limitations of this kind of approach reside in the nature of pharmacological tools, which are not specific enough to parse out the fine molecular mechanisms of translational control that contribute to memory formation. Fortunately, there have been numerous studies in which investigators have utilized genetic models targeting proteins within the mTORC1 signaling pathway; these studies have provided critical information regarding the specific roles of these translational control molecules in synaptic plasticity and memory.

B. FKBP12

1. FKBP12 Structure and Function—FK506-binding proteins (FKBPs) are enzymes with peptidyl-prolyl *cis-trans* isomerase (PPiase) activity. To date, 15 mammalian FKBPs have been found, most of which are expressed in the brain. FKBPs have multiple functions in the nervous system, the best known being a receptor for immunosopressant drugs. These agents act as “prodrug”, and are thus active only when complexed to immunophilins, as is the case with both FK506 and rapamycin, which bind FKBP12[88].

FKBP12 is the smallest member of the FKBP family, with a molecular weight of 12 kDa, and has a minimal peptide sequence that harbors the catalytic domain and a drug-binding pocket. In the absence of a macrolide immunosuppressant, it appears that FKBP12 does not bind to mTOR, but instead is associated with either receptors or Ca^{2+} channels, such as the inositol-(1,4,5)-triphosphate receptor and ryanodine receptors, respectively. When the FKBP12-rapamycin complex is formed, it associates with the FRB domain of mTOR, thereby inhibiting its function (see above)[88,89].

The *FKBP12* gene was deleted in mice to study the function of the protein; however, the resulting deletion led to an embryonic lethal phenotype due to alterations in cardiac development[90]. Consequently, conditional knockout (cKO) mice were generated by crossing a mouse line with floxed alleles of *FKBP12* gene with another line expressing forebrain-specific *Cre-recombinase*. In these mice, the expression of Cre-recombinase mediates a forebrain-specific deletion of the *FKBP12* gene, which occurs approximately 21 days after the birth[80]. The forebrain-specific FKBP12 cKO mice became an invaluable tool to study the influence of FKBP12 on mTORC1, hippocampal synaptic plasticity, and memory consolidation[80].

2. The Role of FKPB12 in Synaptic Plasticity and Memory Consolidation— Surprisingly, the genetic deletion of FKBP12 resulted in increased mTORC1 formation under basal conditions in the absence of rapamycin, suggesting that one of the normal functions of FKBP12 is to suppress mTORC1. In fact, FKBP12 cKO mice exhibited increased phosphorylation at Ser2448 of mTOR, a phosphorylation site that is correlated with activation of mTOR. Furthermore, phosphorylation of S6K1 was enhanced specifically at Thr389, suggesting that activity of the mTORC1 was elevated in the absence of FKBP12. Accordingly, the mTOR-Raptor association also was increased as indicated by enhanced interactions of these proteins in immunoprecipitation experiments in brain tissue from FKBP12 cKO mice[80].

Studies examining synaptic plasticity at Schaffer collateral-CA1 synapses of the hippocampus demonstrated that FKBP12 cKO mice displayed enhanced L-LTP. However, basal synaptic transmission, paired-pulse facilitation, and early phase LTP (E-LTP) were normal in the absence of FKBP12. These results indicate that FKBP12 usually suppresses basal mTORC1 activity and genetic removal of this molecular break enhances L-LTP. Moreover, mTORC1 levels were increased following L-LTP induction in slices from wild-type (WT) mice, but not in slices from FKBP12 cKO mice. The elevated levels of mTORC1 eventually returned to baseline in WT slices; however, mTORC1 levels remained elevated in slices from FKBP12 cKO mice, indicating that there may be an impairment in the downregulation of the mTOR/Raptor interactions in these mice. As expected, the enhanced L-LTP observed in the FKBP12 cKO mice was resistant to rapamycin treatment; however, it was still sensitive to anisomycin. It is interesting to note the presence of an enhancement in the initial phase of E-LTP induction, a stage of LTP that occurs independently of protein synthesis. This observation suggests that FKBP12 also mediates processes that are protein synthesis-independent[80].

In agreement with the enhanced mTORC1 signaling and the increased L-LTP, FKBP12 cKO mice exhibited enhanced formation of contextual fear memory compared to their WT littermates. Additionally, the formation of contextual fear conditioning in WT mice was accompanied by increased mTOR/Raptor interactions. Taken together, these findings suggested that long-term memory is more robust in FKBP12 knockout mice. However, in the novel object recognition (NOR) test, FKBP12 cKO mice exhibited a preference for the familiar, but not novel object. Moreover, in the MWM, spatial learning and memory were normal, but when the platform was moved to a different location, the FKBP12 cKO mice exhibited impaired reversal learning. This type of perseverative behavior was confirmed in a

water-based Y maze[80,91]. These findings suggest that elevated mTORC1 signaling enhance certain types of associative memory, but impair reversal learning, a more demanding cognitive task.

C. 4E-BPs

1. 4E-BP Structure and Function—4E-BPs modulate the formation of the eIF4G initiation complex. 4E-BP normally binds to and sequesters eIF4E, thereby preventing its association with eIF4G and blocking eIF4F formation. When not bound to 4E-BP, eIF4E is free to associate with eIF4G, a molecular event that promotes the formation of eIF4F and cap-dependent translation initiation[61,92,93].

The binding of 4E-BP to eIF4E is regulated by phosphorylation[94,95]. 4E-BP has multiple phosphorylation sites that are regulated by the activity of several kinases, such as extracellular signal-regulated kinase (ERK), phosphoinositide 3-kinase (PI3K) and mTORC1. Phosphorylation of these sites occurs in an ordered, hierarchical fashion: residues Thr37 and Thr46 are phosphorylated first, which primes the phosphorylation of Thr70 and Ser65 is phosphorylated last[94,95]. Phosphorylation at these sites is sensitive to inhibitors of ERK, PI3K and mTORC1, but the identity of the kinase that phosphorylates each individual site has not been established. Nonetheless, it was shown that phosphorylation of Ser65 occurs last and is rapamycin-dependent, suggesting that mTORC1 activity is necessary to inhibit the binding of 4E-BP to eIF4E, thereby promoting cap-dependent protein synthesis[34]. Numerous studies have shown that proper regulation of ERK, PI3K, and mTORC1 are required for protein synthesis-dependent synaptic plasticity, memory formation, and storage[61,92,94,95]. Consistent with this idea, the expression of 4E-BP has been shown to be necessary for normal synaptic plasticity and memory.

2. The Role of 4E-BP in Synaptic Plasticity and Memory Consolidation—There are multiple isoforms of 4E-BPs, but 4E-BP2 is the isoform with the most robust expression in the brain. Therefore, 4E-BP2 KO mice were generated to assess the role of 4E-BP in synaptic plasticity and memory[96–98].

Various forms of synaptic plasticity were studied in Schaffer collateral-CA1 synapses in 4E-BP2 KO mice. A stimulation protocol that usually induces E-LTP in WT hippocampal slices (1 train of HFS) produced an enhanced and sustained L-LTP in 4E-BP2 KO mice. On the other hand, a protocol that typically elicits L-LTP in WT slices (4 trains of HFS) was unable to elicit L-LTP in 4E-BP2 knockout slices. Indeed, the L-LTP that was induced in the 4E-BP2 KO slices decayed to baseline after 2 hours, compared to the sustained L-LTP in WT slices that persisted for over 4 hours. This suggests that there is a reduced threshold to induce L-LTP in 4E-BP2 KO mice. Consistent with this, 4E-BP2 KO mice displayed an increase in eIF4E-eIF4G interactions, which is an index of increased cap-dependent protein synthesis and presumably underlies this enhanced plasticity. Indeed, the sustained plasticity observed in 4E-BP2 KO slices was blocked by anisomycin. Importantly, abalation of 4E-BP2 did not alter basal synaptic transmission. Paired pulse facilitation, as well as PTP, a form of short-term plasticity, also was unaffected in slices from the 4E-BP2 KO mice[96,98].

Hippocampus-dependent memory was assessed in the 4E-BP2 KO mice using the MWM and fear conditioning paradigms. 4E-BP2 KO mice exhibited longer escape latencies during MWM training, and did not exhibit a preference for the target quadrant in the probe trials compared to WT littermates. 4E-BP2 KO mice also exhibited impaired contextual fear conditioning[96–98]. Overall, these results suggest that the relationship between protein synthesis, synaptic plasticity, and memory is very tightly regulated. Specifically, these findings suggest that there is an optimal level of protein synthesis that underlies the coordination between synaptic plasticity and memory, and that exaggerated levels of protein synthesis are detrimental to cognitive function.

D. eIF4E

1. eIF4E Structure and Function—eIF4E, the cap-binding protein promotes protein synthesis by binding to eIF4G. Beside its role in translation regulation, eIF4E is also important for mRNA stability and the proper subcellular localization of mRNA. eIF4E is regulated by phosphorylation via the mitogen-activate protein kinase-interacting serine/threonine kinase 1 and 2 (Mnk1/2). Mnk1/2 is activated by the ERK and p38 mitogen activated protein kinase pathways, and subsequently phosphorylates eIF4E on serine 209. In fact, mice that lack Mnk1/2 did not exhibit phosphorylation of eIF4E at Ser209[99–105].

It has been proposed that Mnk1/2 binds to the scaffolding protein, eIF4G. This molecular event, concomitant with the binding of eIF4E to eIF4G, is required for the subsequent phosphorylation of eIF4E[63,105]. Moreover, it has been shown that phosphorylation of eIF4E reduces its cap-binding affinity[99,101,106]. These data contribute to the formulation of a model in which dephosphorylated eIF4E binds to the mRNA cap structure, promotes eIF4F formation, followed by the recruitment of ribosomes to the mRNA. Subsequently, the phosphorylation of eIF4E reduces its affinity for the cap structure on the mRNA and allows the ribosome to scan for the AUG start codon[63,107,108]. However, evidence regarding the functional effect of eIF4E phosphorylation is contradictory. It has been reported that mice lacking Mnk1/2 are viable and apparently normal, although their synaptic plasticity and memory has not yet been analyzed[109]. On the other hand, it has been shown that pharmacological treatments, such as BDNF and NMDA-R agonists, that induce protein synthesis-dependent synaptic plasticity increase the phosphorylation of eIF4E in an ERK-dependent manner[110,111].

2. The Role of eIF4E in Synaptic Plasticity and Memory Consolidation—The involvement of eIF4E in synaptic plasticity and memory formation has been addressed with 4EGI-1, a compound that specifically inhibits the interaction of eIF4E with eIF4G[112]. Blockade of eIF4E/eIF4G interactions with bath application of 4EGI-1 in hippocampal slices interferes with L-LTP without affecting basal synaptic transmission, PPF and E-LTP. Moreover, the inhibition of L-LTP by 4EGI-1 was accompanied by a reduction in HFS-induced protein synthesis in the slices[113]. These experiments suggest that the activity of eIF4E is necessary for the maintenance of protein synthesis dependent L-LTP. Consistent with the LTP studies, infusions of 4EGI-1 in the amygdala of rats trained in a cued fear conditioning paradigm blocked the consolidation of long-term memory[114]. However, the same infusions administered after memory retrieval did not affect the reconsolidation of

cued fear memory. Moreover, it also was shown that the association of eIF4E with eIF4G was increased during consolidation but not reconsolidation of cued fear memory[114]. These data suggest a different requirement for the formation of the initiation complex eIF4F, and consequently cap-dependent translation, in the consolidation and reconsolidation of cued fear memory.

E. S6K1

1. S6K1 Structure and Function—The *S6K*s are a family of genes that, broadly speaking, are involved in many facets of cellular homeostasis. The dysregulation of S6Ks has been implicated in a variety of pathological states including, but not limited to obesity, cancer, aging, and aberrant metabolism[76,115–117]. The *S6K* genes were discovered during the investigation of the family of kinases that phosphorylate the 40S ribosomal protein S6. One of these genes was originally described as the 70 kDa ribosomal S6K and is now known as p70 S6K1, or simply S6K1[118]. The linking of the S6Ks to the mTORC1 signaling pathway was not discovered until later.

In 1995, a novel phosphorylation site was discovered on S6K1 that was sensitive to rapamycin treatment[119]. Shortly thereafter, *in vitro* kinase assays identified S6K1 to be one of the substrates of mTORC1, along with 4E-BP1[120]. This phosphorylation of S6K1 by mTORC1 then was found to be linked to the phosphorylation of S6.

S6K1 and S6K2 are part of the AGC kinase family, so named after the protein kinase A, G, and C families, and kinase domains with a two-lobe fold structure. Activation of these kinases occurs near the C-terminus C-lobe loop by T-loop kinases. Both S6K1 and S6K2 encode two isoforms, known as S6K1S and S6K1L, and S6K2S and S6K2L. S6K1 is the isoform that has been studied most intensely and whose function is best understood, especially in the nervous system. Similar to many proteins, S6K1 contains multiple phosphorylation sites. However, the most well studied activators of S6K1 are in the insulin/IGF pathway. As mentioned earlier, this pathway activates mTORC1 via PI3K, which is now known to result in the phosphorylation of S6K1 on Thr389[76,115,116]. S6K1 can also be activated in a PI3K-independent manner through Ras/ERK signaling, which results in phosphorylation of Ser421/Ser424[121].

The consequences of S6K1 phosphorylation are many; however, we will focus on the effects that relate to the regulation of protein synthesis. As mentioned, S6K1 phosphorylates S6, eIF4B, as well as eEF2 kinase, suggesting that S6K1 is involved with both the initiation and elongation phases of protein synthesis[95]. It was originally thought that the phosphorylation of S6 by S6K1 was responsible for the translation of TOP mRNAs. However, although multiple studies have questioned the validity of this hypothesis[122,123], the involvement of S6 phosphorylation in the translation of TOP mRNAs remains a possibility[124]. The phosphorylation of eIF4B functions to enhance the helicase activity of eIF4A, a component of eIF4F, which helps unwind complex 5′ UTR structures. S6K1 also phosphorylates an eIF4A inhibitor, PDCD4, which enhances the helicase activity of eIF4A[95]. Finally, S6K1 phosphorylates and inhibits eEF2 kinase, which is responsible for the phosphorylation of eEF2. In this manner, S6K1 indirectly regulates eEF2 phosphorylation and ribosomal

translocation in peptide chain elongation[67]. Thus, S6K1 also plays a role in the elongation phase of protein synthesis.

2. The Role of S6K1 in Synaptic Plasticity and Memory Consolidation—Genetic deletion of both S6K1 and S6K2 is embryonic lethal; however single mutants are born at traditional Mendelian ratios[122]. S6K1 and S6K2 KO mice were utilized to study synaptic plasticity and memory[125]. S6K1 and S6K2 knockout did not exhibit any major impairments in either basal synaptic transmission or synaptic plasticity. The S6K1 and S6K2 KO mice exhibited a mild impairment in the acquisition phase of the fear conditioning[125]. For contextual fear memory, S6K1 KO mice exhibited deficient short-and long-term memory; however, S6K2 KO mice did not exhibit impairments in memory until 7 days after training[125]. These deficits also were observed in the S6K1 and S6K2 heterozygous KO mice[125]., suggesting that full expression levels of these proteins are required for proper contextual fear memory.

S6K1 and S6K2 KO mice were tested for CTA, another associative learning task, and latent inhibition of CTA[125]. In the CTA task, the S6K1 KO mice displayed a lower aversion index than their WT littermates, and the extinction of this memory indicated that the S6K1 KO mice rapidly extinguished the association between the saccharin and LiCl injection[125]. Together, these results suggest that CTA memory is weak in S6K1 KO mice. In contrast, S6K2 KO mice did not exhibit impaired CTA; however, the latent inhibition of CTA, was significantly higher than their WT littermates, suggesting that S6K2 is required for novel taste experiences.

The MWM was used to assess the spatial learning and memory abilities of the S6K1 and S6K2 KO mice. During a probe trial on day 3, the S6K1 KO mice exhibited fewer platform crossings and spent less time in the target quadrant; this effect was not observed in the day 7 probe trial. The S6K2 KO mice did not exhibit impairments in the MWM[125].

In summary, S6K mutant mice did not display learning and memory impairments, and to date, a major role for these kinases in LTP and memory have not been demonstrated.

F. eIF2 and eIF2α Kinases

1. eIF2 Structure and Function—eIF2 is composed of three subunits, α, β and γ, and phosphorylation of the α subunit at serine 51 (Ser51) converts eIF2 into an inhibitor of eIF2B, the GEF that catalyzes the conversion of inactive eIF2-GDP into active eIF2-GTP. Thus, phosphorylation of Ser51 on eIF2α impairs eIF2 recycling by inhibiting eIF2B such that it remains blocked in an inactive eIF2-GDP state. Even if the relative abundance of eIF2/eIF2B has not been reported in the nervous system, in other tissues it has been shown that eIF2 is more highly expressed than eIF2B, and phosphorylation of a small fraction of eIF2α is sufficient to inhibit eIF2B and blocks general protein synthesis[35,52].

Phosphorylation of eIF2α at Ser51 is a highly dynamic and regulated event that is mediated by four different kinases and two phosphatases. The kinases that phosphorylate eIF2α are protein kinase-RNA regulated (PKR), heme-regulated initiation factor 2α kinase (HRI), eIF2α kinase 3 (PERK), and general control non-derepressible 2 (GCN2). All of these

kinases are present in the brain and are activated by different cellular stressors: PKR is activated by double-stranded RNA, HRI is induced by low heme levels, PERK is activated by endoplasmic reticulum (ER) stress as well as unfolded proteins in the ER, and GCN2 is induced by amino acid starvation[52]. Although all of the eIF2α kinases are present in the brain, GCN2 appears to be the most abundant, consistent with the indication that GCN2 mRNA is strongly expressed in the mouse brain[126,127].

The phosphatase complexes that dephosphorylate eIF2α are the complex of the eIF2α-specific regulatory subunit constitutive repressor of eIF2α phosphorylation (CReP) and protein phosphatase I catalytic subunit (PPIc), and the complex of growth arrest and DNA damage-inducible gene 34 (GADD34) and PPIc. The regulatory subunits CReP and GADD34 provide specificity for the complex toward eIF2α[128].

eIF2α kinases historically have been described as stress-responsive regulators of general protein synthesis. Thus, phosphorylation of eIF2α on Ser51 suppresses general protein synthesis but at the same time stimulates translation of a subset of mRNAs that contain an open reading frame (ORF) in the 5′-UTR[129,130]. This type of gene-specific translational control has been reported for the transcriptional activator GCN4 in yeast[131,132] and for activating transcription factor 4 (ATF4, also known as CREB2) in mammalian cells[133,134]. The increase in ATF4 that results from increased eIF2α phosphorylation is negatively correlated with CREB-dependent transcription, synaptic plasticity, and memory (see below).

Several studies have been performed that indicate an important role for eIF2α phosphorylation in memory formation. We will describe studies that have performed with GCN2 KO mice, PERK cKO mice, PKR KO mice, and eIF2α^{+}/S51A mutant mice.

2. GCN2—The first studies dissecting the role of eIF2α phosphorylation in synaptic plasticity and memory were performed in mice with a constitutive deletion of GCN2, the eIF2α kinase that is most robustly expressed in the brain[135].

Various forms of hippocampal synaptic plasticity were studied in GCN2 KO mice. A stimulation protocol that usually induces E-LTP in WT hippocampal slices (1 train of HFS) produced an enhanced and sustained L-LTP in GCN2 KO mice. Induction of E-LTP in GCN2 KO mice resulted in long-lasting LTP, which was dependent on transcription, translation, as well as cAMP-dependent protein kinase activity. Moreover, anisomycin and actinomycin D, inhibitors of translation and transcription, respectively, also decreased the potentiation during the early phase of LTP. Overall, these results suggest that there is a reduced threshold for LTP induction in the GCN2 KO mice that is sensitive to inhibitors of protein synthesis and transcription. In addition, L-LTP was studied in the GCN2 KO mice by using electrical (4 trains of HFS) and chemical stimulation (bath application of forskolin) protocols. In these conditions, L-LTP decayed to baseline after 3 hours in GCN2 KO slices, whereas in WT slices, L-LTP persisted over 3 hours. Despite the abberant synaptic plasticity observed in various forms of LTP in GCN2 KO mice, the mice did not exhibit any differences in basal synaptic transmission compared to their WT littermates[135,136].

Biochemical analysis in WT slices following induction of L-LTP showed a decrease in GCN2 and eIF2α phosphorylation levels. However, these biochemical effects were not observed following the induction of E-LTP. Thus, the decrease in GCN2 activity and eIF2α phosphorylation are elicited following strong stimulation, but not weak stimulation, consistent with a role for protein synthesis in L-LTP, but not E-LTP. In agreement with these results, basal levels of phosphorylated eIF2α and ATF4 were reduced in GCN2 KO mice. Interestingly, anisomycin and actinomycin D both block the long-lasting LTP induced in the GCN2 KO mice with a weak stimulation protocol, suggesting that there may be an enhancement in the translation of pre-existing transcripts, as well as transcription of mRNA, in the GCN2 KO mice[135,136].

In behavioral studies of the GCN2 KO mice, a deficit in contextual fear conditioning, but not cued fear conditioning, was observed. In the MWM, WT mice were able to learn and remember the platform position using the standard training protocol (3 training sessions/day), but the GCN2 KO mice were slower to learn and remember the position of the platform. Based on the observation that weak electrical stimulation led to L-LTP, the GCN2 KO mice were trained in the MWM using a weak training protocol (1 session per day), which revealed shorter escape latencies in the GCN2 KO mice, as well as increased time spent in the target quadrant on day 5 of training. Thus, the spatial memory of the GCN2 knockout mice was enhanced following weak training[135]. Overall, these behavioral studies indicate that the role of GCN2, both in synaptic plasticity and memory, is tightly regulated, and most likely linked to the regulation of optimal levels of protein synthesis during these processes.

3. eIF2α S51A—The studies with the GCN2 KO mice suggested that changes in eIF2α phosphorylation play a significant role in synaptic plasticity and memory consolidation. To more directly study the role of eIF2α phosphorylation in synaptic plasticity and memory, a mutant mouse was generated with a point mutation on the eIF2α phosphorylation site. The mice were heterozygous mutants where Ser51 was mutated to an alanine (S51A), and exhibited an approximate 50% reduction in eIF2α phosphorylation[137].

Similar to the previous studies performed in GCN2 KO mice, these eIF2α S51A mutant mice are expected to exhibit L-LTP using a weak stimulation protocol. Consistent with this expectation, 1 train of HFS resulted in a sustained L-LTP in eIF2α mutant mice. This long-lasting LTP was blocked by anisomycin, as well as by actinomycin D, demonstrating the requirement of both translation and transcription. The eIF2α S51A mutant mice did not exhibit any differences in basal synaptic transmission. Interestingly, the eIF2α mutants exhibited stable L-LTP using both electrical and chemical stimulation protocols, which was not observed in GCN2 KO mice (see above)[137].

Because of the previous studies that indicated that GCN2 KO mice were able acquire knowledge of the platform position in the MWM using a weak training protocol, the eIF2α S51A mutant mice were tested on the MWM using a similar weak training protocol. eIF2α S51A mutant mice were trained once a day for 6 days on the MWM and by day 6, the mutant mice exhibited a shorter escape latency and spent a greater amount of time in the target quadrant compared to WT littermates. However, using the standard MWM training

protocol, eIF2α S51A mutant mice also demonstrated shorter escape latency during training compared to their WT littermates. In the probe test, eIF2α mutants also spent more time in the target quadrant compared to their WT littermates. Thus, although GCN2 KO mice showed impairments in the MWM using standard training protocols, these effects were not observed in the eIF2α S51A mutant mice[137]. These results indicate that decreased eIF2α phosphorylation leads to enhanced hippocampus dependent learning and memory, regardless of the strength of the training protocol.

Consistent with results of the MWM studies, the eIF2α S51A mutant mice also were tested on contextual and auditory fear conditioning and showed enhanced memory despite the use of differing footshock intensities. In addition, in the conditioned taste aversion (CTA) task, eIF2α S51A mutant mice did not exhibit a difference in the aversion index to sucrose compared to WT mice; however, latent inhibition of CTA in the mutant mice was decreased compared to their WT littermates[137]. These differing outcomes may be a result of ceiling effects in the CTA task that are unmasked in the latent inhibition of CTA.

The results of the behavioral studies of the eIF2α S51A mutant mice suggested that inhibiting the dephosphorylation of eIF2α would inhibit L-LTP and memory formation. Thus, Sal003, an inhibitor of eIF2α phosphatases, was used to assess the influence of inhibiting the dephosphorylation of eIF2α on synaptic plasticity and memory. As expected, inhibiting the dephosphorylation of eIF2α in WT hippocampal slices led to an impairment in L-LTP. Sal003 did not have this effect in slices from ATF4 KO mice, suggesting that the Sal003-mediated impairment in L-LTP is ATF4-dependent. The effect of Sal003 in blocking L-LTP was specific to LTP induction, as the application of this compound 45 min after LTP induction had no effect on potentiation[137].

Contextual fear conditioning experiments in WT mice demonstrated that eIF2α phosphorylation was reduced 30 minutes after training. Thus, blocking this dephosphorylation would be expected to interfere with contextual fear memory. Indeed, Sal003 infusion into the dorsal hippocampus blocked the formation of contextual fear memory. Moreover, using a standard training protocol in the MWM, Sal003 infusion also blocked the formation of long-term memory as evidenced by an increase in escape latency and reduced target quadrant occupancy[137].

The results of the experiments performed in the eIF2α S51A mutant mice confirmed observations made in the GCN2 KO mice. Specifically, that the phosphorylation and dephosphorylation of eIF2α is strictly regulated during various forms of LTP and various types of memory tasks.

4. PERK—PERK is an eIF2α kinase that is activated during ER stress to reduce protein synthesis. Similar to GCN2, the phosphorylation of eIF2α by PERK leads to the translation of ATF4. Forebrain-specific PERK cKO were generated and tested on several different behavioral paradigms to measure learning and memory[138]. The PERK cKO mice exhibited an enhanced preference for the novel object in the NOR task. PERK cKO mice also exhibited an impairment in the extinction of cued fear conditioning. In the MWM, PERK cKO mice did not exhibit any differences in learning the position of the platform, and

performed similar to WT during probe tests. However, the PERK cKO were impaired in learning the location of the platform when the position of the platform was switched to the opposite quadrant[138]. These results suggested that the PERK cKO mice exhibit perseverative behavior. The perseverative behavior of the PERK cKO mice was confirmed by examining reversal learning a water-based Y-maze task, an additional task used to measure cognitive inflexibility. Again, the PERK cKO mice were unable to acquire the location of the new platform position[138]. WT mice were sacrificed to measure levels of eIF2α phosphorylation during reversal learning and a decrease in eIF2α phosphorylation was observed[138], suggesting that the dephosphorylation of eIF2α is required for reversal learning. GCN2 KO mice also were tested for reversal learning in the Y-maze, but they did not exhibit an impairment in this task. The results of these studies suggest that although eIF2α phosphorylation is decreased during reversal learning, the pool of eIF2α that is dephosphorylated is normally phosphorylated by PERK and not other eIF2α kinases.

5. PKR—PKR is an eIF2α kinase that is activated by double stranded RNA (dsRNA) species during viral infection[139]. PKR is another regulator of protein synthesis via its ability to phosphorylate eIF2α; however, another function of PKR is to regulate protein levels via degradative mechanisms, which is a process that is critically involved in synaptic plasticity and memory[140]. LTP and memory function was studied in a PKR cKO in which the levels of PKR were increased in the CA1 region of the hippocampus via chemical induction[141]. Hippocampal synaptic plasticity was also studied in the PKR KO mice and E-LTP was found to be similar to their WT littermates. However, L-LTP induced with TBS was impaired in the PKR KO mice, suggesting that PKR is required for this form of synaptic plasticity[141]. Moreover, PKR KO mice exhibited impairments in contextual fear conditioning, but not auditory fear conditioning, suggesting that the fear memory deficit was hippocampus-dependent[141]. Importantly, the PKR KO mice do not exhibit any reduction in general translation[141], indicating that the L-LTP and contextual fear memory impairments are due to reduced eIF2α phosphorylation causing gene-specific changes in translation in these mice.

Collectively, the vast array of studies performed in eIF2α kinase (GCN2, PERK and PKR) mutant mice and in the eIF2α S51A mutant mice clearly demonstrate that the regulation of eIF2α phosphorylation is involved in long-lasting synaptic plasticity and several types of long-term memory. Specifically, there is an optimal level of eIF2α phosphorylation that must be achieved for these different forms of synaptic plasticity and memory, and that the mechanisms involved in the control of eIF2α phosphorylation are not conserved across various experimental paradigms.

IV. Concluding Remarks

Today we have a substantial understanding of the molecular mechanisms, including the regulation of translation, underlying long-term memory formation and the importance of the synaptic changes that are required for it. However, many of the molecular details of the regulation of protein synthesis are still missing and we are just now beginning to understand how translation is locally regulated.

Although, synaptic plasticity is a key step in memory storage, it is becoming more apparent that a simple enhancement in the efficacy of a synapse is not sufficient to form and store a complex memory. It is believed, instead, that changes in synaptic strength must occur within a subset of neurons to produce a specific alteration in information flow through a neural circuit. How translation impacts synaptic changes that ultimately affect the neuronal circuitry is currently under investigation.

Finally, there is compelling evidence that defects in memory storage result from pathological changes in the fundamental mechanisms that control molecular signaling, including translational control, and synaptic plasticity. A future challenge that remains is to understand how the molecular mechanisms that control translation during long-lasting synaptic plasticity and memory formation are impaired with age or disease, such as in Alzheimer's disease, neuropsychiatric disorders, and neurodevelopmental disorders. Thus, it is of critical importance to understand in greater detail, the molecular mechanisms that are required for memory formation as well as the abnormal changes that take place in neuropathological conditions in order to design specific therapeutic compounds that could be used to restore cognitive function.

V. References

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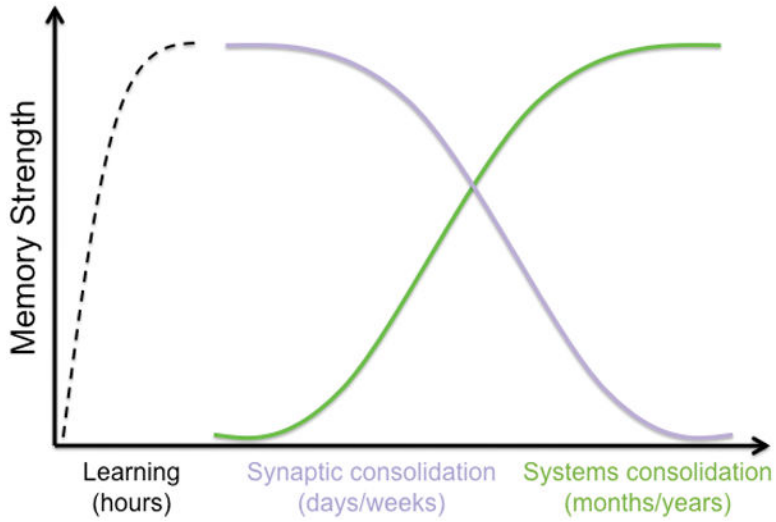

Figure 1. The reorganization of memory occurs with the passage of time

Learning occurs within hours and is initially stored in local synaptic circuits. This "synaptic consolidation" persists for days and up to weeks. Synaptic consolidation is gradually converted to systems consolidation, where the memory can persist for years, in broadly distributed cortical networks.

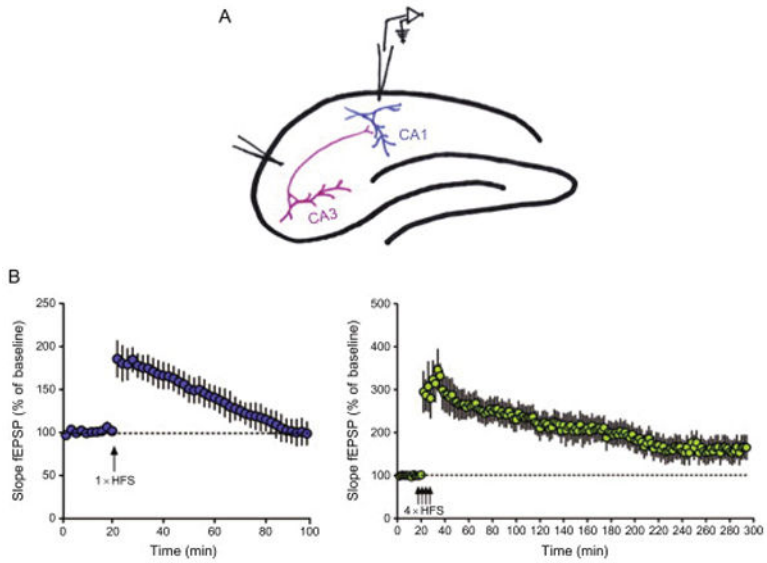

Figure 2. LTP: A cellular substrate for memory formation

A) Hippocampal LTP can be induced by high-frequency stimulation of the Schaffer Collateral pathway. Shown is a diagram of a stimulating electrode, which is placed near the axonal tract originating from CA3 pyramidal neurons, and a recording electrode, which is placed in the dendritic tree of CA1 pyramidal neurons. *B) E-LTP and L-LTP induction is achieved using high-frequency stimulation.* Early phase LTP (E-LTP) is induced by 1 train of high frequency stimulation (HFS) and long-lasting, late phase LTP (L-LTP) is induced using 4 spaced trains of HFS. E-LTP persists approximately 1 hour, whereas L-LTP persists for several hours.

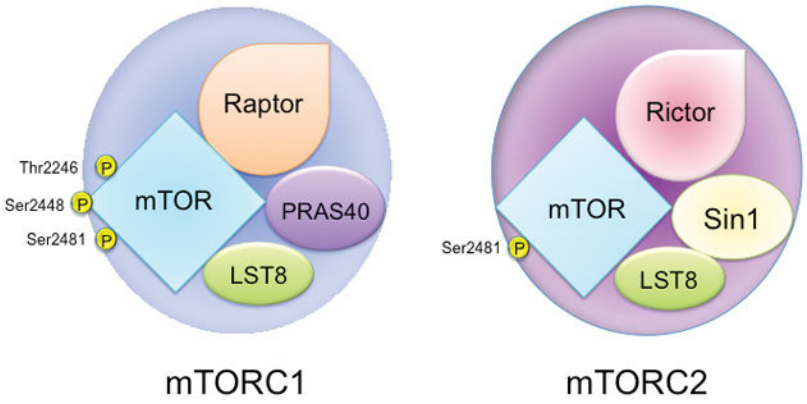

Figure 3. mTOR exists in two complexes

mTORC1 is comprised of mTOR, raptor, LST8 and PRAS40. mTORC2 is comprised of mTOR, rictor, LST8 and sin1.]

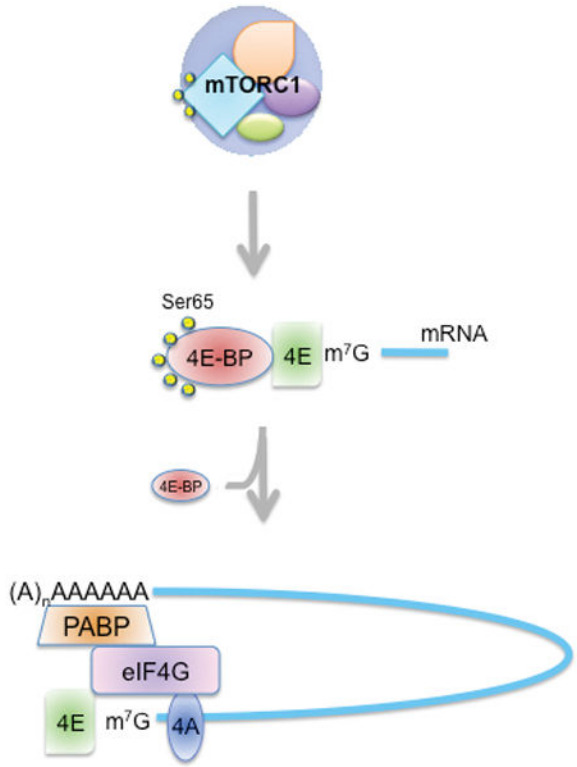

Figure 4. The phosphorylation of 4E-BP by mTORC1 leads to the formation of eIF4F, the cap-dependent translation initiation complex

The eIF4 group of eIFs form the cap-binding complex eIF4F, which consists of the cap-binding protein eIF4E, the DEAD-box RNA helicase eIF4A, and the scaffolding protein eIF4G. eIF4G circularizes the mRNA by interacting simultaneously with eIF4E and PABPs.