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# The role of protein synthesis in memory consolidation: Progress amid decades of debate

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## Abstract

A major component of consolidation theory holds that protein synthesis is required to produce the synaptic modification needed for long-term memory storage. Protein synthesis inhibitors have played a pivotal role in the development of this theory. However, these commonly used drugs have unintended effects that have prompted some to reevaluate the role of protein synthesis in memory consolidation. Here we review the role of protein synthesis in memory formation as proposed by consolidation theory calling special attention to the controversy involving the non-specific effects of a group of protein synthesis inhibitors commonly used to study memory formation *in vivo*. We argue that molecular and genetic approaches that were subsequently applied to the problem of memory formation confirm the results of less selective pharmacological studies. Thus, to a certain extent, the debate over the role of protein synthesis in memory based on interpretational difficulties inherent to the use of protein synthesis inhibitors may be somewhat moot. We conclude by presenting avenues of research we believe will best provide answers to both long-standing and more recent questions facing field of learning and memory.

## Keywords

Protein synthesis; RNA synthesis; Consolidation; Memory; Amnesia; Reconsolidation; Protein synthesis inhibitor; Sleep; Gene expression; Transcription; Translation; Knockout; Synaptic; Systems; Side effects; Rescue; Hippocampus; Learning; LTP; cAMP; CREB; NMDA; Tagging; Post-translational modification

## 1. Introduction

I sometimes feel, in reviewing the evidence on the localization of the memory trace, that the necessary conclusion is that learning is just not possible (Lashley, 1950).

One of the most puzzling questions facing psychologists and neurobiologists alike is one that was posed centuries ago: what is the nature of memory? How does wakeful experience alter neural circuits within the brain in such a precise and meaningful way that even decades later

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we are able to invoke a remarkably detailed percept of our own history? Indeed, the formation of cognitive associations between external stimuli or between our actions and their consequences can be demonstrated with relative ease. However, it is considerably more difficult to causally connect cellular and molecular events to the instantiation of such associations. Nevertheless, we are now equipped with sophisticated molecular and genetic techniques that afford us the opportunity to probe deeper than ever before into the molecular underpinnings of memory.

In the first section of this review, we examine the emergence of consolidation theory—the idea that memories are stabilized over time—recalling several important findings that were seminal to its development and continuing evolution. We then examine the basis of the long-standing debate regarding the validity of a major tenet of consolidation theory: that new proteins must be synthesized to stabilize newly acquired memories. Indeed, this debate, although ignored by many, has never been resolved to the satisfaction of some. We then briefly summarize the remarkable progress that has been made in understanding consolidation in spite of this debate and present promising new approaches being developed to address some old questions as well as questions that have arisen along the way. Lastly, we conclude by addressing a few alternatives or addendums to consolidation theory that merit consideration.

## 2. The emergence of consolidation theory: The role of studies using protein synthesis inhibitors

Early empirical forays investigating memory function began in 1878 when Hermann Ebbinghaus introduced the concept of “retroactive interference”. Using sequentially memorized lists of nonsense syllables, Ebbinghaus showed that “forgetting” could be attributed to the interfering effects of subsequently learned matter (Ebbinghaus, 1885), thereby establishing the existence of temporal constraints on memory formation and storage. In 1900, using improved methodology and controls, Müller and Pilzecker confirmed that memory for verbal material was susceptible to disruption if new material was introduced too soon after the initial acquisition period. Thus, they proposed that new memories required a period of “consolidation” to fixate or become resistant to disruption (Lechner, Squire, & Byrne, 1999; Müller & Pilzecker, 1900).

Support for Müller and Pilzecker’s consolidation theory came a half-century later when it was observed that memory in rats could be retroactively disrupted by applying an electroconvulsive shock near the time of training (Duncan, 1949) or through head injuries involving the hippocampus and related structures (Russell & Nathan, 1946). In both forms of retrograde amnesia, memory loss varies inversely with the age of the memory where new memories are more susceptible to disruption. The amnesia described by Russell and Nathan, which can extend for years prior to the actual neural insult, led to the hypothesis that memories are formed and stored in the hippocampus temporarily but are then transferred to distal cortical sites for permanent storage. This relatively slow process is now referred to as *systems consolidation* (McGaugh, 2000; Squire & Bayley, 2007). This is in contrast to the relatively faster processes of stabilization revealed by verbal interference, electroconvulsive shock, and pharmacological experiments (described below) thought to occur on a cellular or synaptic or level (McGaugh, 1966). Parenthetically, the precise time course during which synaptic consolidation occurs is unclear but has been reported to range anywhere from 500 ms to hours depending on the type of memory being examined, the training procedures, and the amnesic agent used to probe memory (Miller & Matzel, 2006).

This review focuses primarily on the role of protein synthesis during synaptic consolidation largely because the vast majority of cellular and molecular research has targeted the more accessible processes occurring immediately after novel learning situations. However, the

existence of systems consolidation must also dictate to an equal extent how we envision and study memory storage over extended periods of time (Frankland & Bontempi, 2005).

Another major step in the evolution of consolidation theory that changed the way the field would conceptualize the consolidation of memory on a cellular level also occurred in 1949 when Hebb introduced his “dual-trace hypothesis” of memory formation. Hebb proposed that reverberation of activity within assemblies of neurons was the essence or trace of short-term memory and that if maintained long enough some growth processes at the level of the synapse could lead to long-term memory (Hebb, 1949). Indeed, disruptions in neuronal reverberation was seen as an attractive explanation of the mechanism by which retrograde amnesia might occur (Glickman, 1961; McGaugh, 1999; Misanin, Miller, & Lewis, 1968; Schneider & Sherman, 1968).

Further support for consolidation theory was offered in the late 1950s, after Scoville and Milner described the memory deficits experienced by the famous patient H.M. After bilateral resection of the medial structures of the temporal lobe to treat epilepsy, it was evident that H.M. had severe short-term memory deficits, unable to form new hippocampus-dependent long-term memories (Scoville & Milner, 1957). Importantly, when tested in delayed matching and delayed comparison tasks, H.M.’s performance worsened as the delays increased leading Milner to conclude, in support of Hebb’s view of consolidation, that a distinction exists between the initial processing of memory that decays rapidly and a later, secondary process that is responsible for long-term storage of information (Milner, 1972).

Yet, while great progress was being made in understanding memory from observations from both human and animal studies, direct evidence of the mechanisms by which experience produced long-lasting neural changes was still lacking. This would all change when, in considering how memory traces might be instantiated within neural circuitry on a molecular level Monné, as well as Katz and Halstead, hypothesized that protein molecules were somehow required (Katz & Kalstead, 1950; Monné, 1948; Sutton & Schuman, 2006). At last, after a decade or so of speculation, Flexner and colleagues demonstrated memory for a discriminative avoidance task (performed in a Y-maze) could be disrupted in mice using the protein synthesis inhibitor puromycin (Flexner, Flexner, Stellar, De La Haba, & Roberts, 1962; Flexner, Flexner, & Stellar, 1963). Additionally, some cortical specificity in memory formation was evident in that only bilateral temporal injections affecting the hippocampus and adjacent temporal cortex consistently disrupted memory for recently acquired memories. Consistent with the notion of systems consolidation, temporal infusions in combination with infusions more rostral and caudal were required to disrupt more remote memories (e.g., 11–43 days old), indicating the establishment of a more distributed trace over time (Flexner et al., 1963). A flurry of reports investigating the role of a variety of protein synthesis inhibitors on different memory tasks soon followed (Agranoff, 1967; Agranoff, Davis, & Brink, 1965; Agranoff & Klinger, 1964; Barondes & Cohen, 1966; Barondes & Cohen, 1967; Davis & Squire, 1984; Dudai, 2004; Flexner & Flexner, 1966; Flexner, Flexner, & Roberts, 1966; Flexner, Flexner, & Stellar, 1965; McGaugh, 2000). It should also be noted that other pharmacological treatments were found to facilitate rather than disrupt memory (Doty & Doty, 1966; Hunt & Krivanek, 1966; McGaugh & Petrinovich, 1965; Pare, 1961; Stratton & Petrinovich, 1963).

In general, it was found that protein synthesis inhibitors, like electroconvulsive shock, were most efficacious when administered around the time of training (but see below for exceptions) having little effect on established memories. Therefore, consolidation has historically been assumed to occur but once, stabilizing the engram permanently. However, this notion too is currently under scrutiny for processes such as multiple “waves” of consolidation (Bourtchouladze et al., 1998; Freeman, Rose, & Scholey, 1995; Scholey, Rose, Zamani, Bock,

& Schachner, 1993; Stork & Welzl, 1999) and reconsolidation (Alberini, 2005; Dudai, 2006; Dudai & Eisenberg, 2004; Nader, 2003; Tronson & Taylor, 2007).

Given the central dogma of molecular biology at that time, specifically that DNA is transcribed to synthesize RNA which is translated to synthesize protein, the ability of RNA synthesis inhibitors to disrupt memory quickly followed the Flexners' initial studies using protein synthesis inhibitors. Early studies using the RNA synthesis inhibitor actinomycin D produced mixed results. For example, actinomycin D successfully impaired memory in goldfish when infused immediately after training but not after a 1-h delay (Agranoff, Davis, Casola, & Lim, 1967) suggesting the synthesis of new proteins subsequent to learning-induced transcription may be important in memory formation. However, in rodents, systemic administration of actinomycin D was extremely toxic complicating the interpretation of these studies (Squire & Barondes, 1970). Nevertheless, later studies would provide a better demonstration of the requirement of transcription in memory formation as summarized below (Igaz, Vianna, Medina, & Izquierdo, 2002).

Most importantly these early studies demonstrating the involvement of *de novo* protein synthesis in memory formation made tangible the notion that the physical basis of memory lies in the learning-related growth or remodeling of synaptic connections in a protein synthesis-dependent fashion. However, this notion was not universally accepted.

### 3. Putting the breaks on translation: Non-specific effects of protein synthesis inhibitors on memory

By providing the first experimental evidence of a *protein synthesis-dependent* stage of memory formation, the Flexners catalyzed the incorporation of protein synthesis into the very definition of consolidation. Ironically, it was also the Flexners who incited the ongoing debate regarding the actual role of *de novo* protein synthesis in memory formation. Adding to the irony is that this theoretical "about face" was sparked after Flexner and colleagues demonstrated that an injection of *saline* could recover memory thought to be destroyed by puromycin (Flexner & Flexner, 1967). Although the mechanism by which saline restored memory was a mystery, it was nevertheless concluded that puromycin only blocked the *expression* of memory by acting on cellular mechanisms not related to protein synthesis. However, it was soon discovered that saline was only able to restore memory when puromycin was administered 24 h or more after training; puromycin infusions given immediately before or after training resulted in memory impairments that could not be restored (Flexner & Flexner, 1968a).

#### 3.1. Effects of altered catecholamine function and pharmacological rescue of amnesia

Suspicions that protein synthesis inhibitors had serious side effects grew after it was shown that puromycin inhibited the increase in norepinephrine synthesis normally observed after stimulation of the hypogastric nerve of the vas deferens in the guinea-pig (Weiner & Rabadjija, 1968). Reduced norepinephrine levels were also known to temporarily impair performance on conditioned avoidance responses (Schoenfeld & Seiden, 1969; Seiden & Peterson, 1968). An experiment performed by Flexner and colleagues then tested a variety of drugs on their ability to restore memory in mice that were injected bitemporally with puromycin 24 h after training in the Y-maze. Memory in a few instances was found to be at least partially restored by drugs that act on the adrenergic system (e.g., reserpine or L-DOPA). Therefore, the authors hypothesized that the adsorption of puromycin to adrenergic sites might underlie memory impairments rather than the inhibition of translation (Roberts, Flexner, & Flexner, 1970). It should be noted that puromycin in this study was not administered during the critical period immediately after acquisition during which memory is thought to depend most on protein synthesis. Thus, these drug treatments could have been acting to enhance some aspect of less

severely disrupted memories. Furthermore, it should be noted that the drug treatments used in the above study were administered to a very limited number of mice and killed as many as had experienced full or partial memory restorations.

Subsequent evidence demonstrated that a variety of protein synthesis inhibitors decreased tyrosine hydroxylase activity, thereby altering endogenous catecholamine levels (Flexner & Goodman, 1975; Flexner, Serota, & Goodman, 1973). Of interest, is a study by Flexner and colleagues that examined the effect of infusions of the predominately  $\alpha 1$ -adrenergic agonist metaraminol on acetoxycycloheximide-induced amnesia 24 h post-training in rats. Bitemporal injections of acetoxycycloheximide were administered 5 h *prior* to training in a Y-maze, a procedure known to only cause *transient* amnesia. Metaraminol boosted performance levels when administered 0.5 h before training, 0–2 h after training, or 0.5–2 h before testing. Metaraminol infusions administered from 2.5–21.5 h after training had no significant effect. The authors suggest that acetoxycycloheximide produced amnesia not by inhibiting protein synthesis but by lowering the amount of norepinephrine available at the time of training (Serota, Roberts, & Flexner, 1972). Indeed, this study has been cited (Routtenberg & Rekart, 2005) as a clear demonstration that protein synthesis inhibitors act to impair memory through mechanisms other than by inhibition of translation. However, the performance improvements observed after metaraminol infusions around the time of training or testing imply that *encoding* and *retrieval* mechanisms were enhanced, respectively. The lack of effect of metaraminol in the 2.5–21.5 h group is consistent with the fact that the potency of the drug had diminished enough over the 2.5 h delay between the end of drug administration and memory testing such that retrieval was no longer enhanced. Indeed, it could be argued that this study actually *supports* consolidation theory inasmuch as the drug had no effects after administration between 2.5 and 21.5 h—a period of time when receptor agonism might not be expected to affect memory consolidation. Importantly, the inhibition of protein synthesis is known not to impair encoding or retrieval (Davis & Squire, 1984). Thus, it seems that metaraminol did not rescue amnesia by reversing the effects of acetoxycycloheximide but overcame them perhaps by enhancing the encoding and retrieval of the weakly consolidated memory. Conceptually, these studies suggest that a single agent might affect disparate cellular processes (e.g., protein synthesis or neurotransmitter function) by interfering with temporally and biochemically distinct aspects of memory processing (e.g., encoding versus consolidation or retrieval). In the case of puromycin, consolidation seems to be impaired by protein synthesis inhibition and retrieval through interference with neurotransmitter function. However, this lesson is often overlooked even today.

Others involved in this debate argued for the role of translation in consolidation, perhaps more convincingly. For example, Squire and colleagues found that repeated injections of  $\alpha$ -methyl-*p*-tyrosine, an inhibitor of tyrosine hydroxylase, left long-term memory unaffected in spite of the fact that brain tyrosine hydroxylase activity was inhibited to levels equal to or surpassing that caused by doses of cycloheximide or anisomycin used to impair memory (Squire, Kuczenski, & Barondes, 1974). A further study by Flood and colleagues compared the effects of anisomycin with the effects of three catecholamine synthesis inhibitors across seven different behavioral paradigms. They reported that pre-trial infusions of any of the four drugs impaired passive avoidance learning but only when training was weak. However, only anisomycin impaired learning when infused *post-trial* during the consolidation period (Flood et al., 1986).

Also relevant to this discussion is a study conducted by Squire and Barondes where they examined the effects of cycloheximide on memory for a shock-escape object discrimination task at a time when 95% of brain protein synthesis was inhibited (1 min, ~95% inhibition at time of training) or after inhibition had been established for significant period of time (118 min, ~70% inhibition at time of training). Mice injected 1 min before training acquired the task



but did not retain the memory when tested the next day. In contrast, mice injected with the same dose of cycloheximide 118 min before training (or 10 min after training) exhibited normal retention (Squire & Barondes, 1976). Since cycloheximide elevates tryrosine levels by 50% from 30 min to 4 h after injection (Flexner et al., 1973) then the accumulation would be expected to be greater in the 118 min group and therefore disrupt memory to a greater degree relative to the 1 min group. However, only the 1 min treatment caused amnesia for the task. Similarly, since alterations in tyrosine hydroxylase activity and catecholamine synthesis develops over 2–3 h after injection the same argument can be made that these effects should be greatest in the group that produced no memory disruptions. The same study also suggests that protein synthesis inhibitors do not cause amnesia by blocking the replacement of constitutive proteins (even those with a half-life of as little as 11 min) but do so by blocking the induction of new proteins needed for memory formation. Taken together, these findings demonstrate that altered tyrosine hydroxylase activity and catecholamine levels, at least on their own, cannot explain the amnesia induced by protein synthesis inhibitors.

Since the Flexners' early memory restoration studies, a variety of drugs (e.g., amphetamine, nicotine, fluoxetine, etc.) have been found to enhance performance in memory tasks even while animals are under the influence of various protein synthesis inhibitors. Because they do so without diminishing levels of protein synthesis inhibition, the apparent "rescue" of amnesia by these pharmacological agents has been cited as further evidence against the requirement of *de novo* protein synthesis in memory formation. However, drug treatments that seem to rescue the memory impairing effects of protein synthesis inhibitors could do so by acting on parallel cellular mechanisms that promote protein synthesis thereby *overcoming* the effects of protein synthesis inhibitors (Davis & Squire, 1984; Gold, 2006) or by modulating other mechanisms on which memory formation depends (e.g., metaraminol). Some drugs enhance memory by mimicking the brain's natural response to arousing or stressful situations (i.e., through the release of epinephrine, glucose, or corticosterone) (Gold, 2003; Gold, 2005; McGaugh, 1983). Importantly, the fact that certain lesions (e.g., adrenalectomy) only prevent the endogenous enhancement of memory (i.e., leaving basic memory intact) demonstrates the notion that certain drugs do not truly rescue amnesia but act to enhance the residual memory that typically exists after various amnesic treatments (Gold, 2006; Roozendaal, Carmi, & McGaugh, 1996; Roozendaal & McGaugh, 1996a; Roozendaal & McGaugh, 1996b). Alternatively, some drugs (in particular drugs of abuse) might act to enhance performance by over-stimulating the very same signaling pathways recruited during experience-dependent learning leading to a type of maladaptive "hijacking" of learning and memory systems (Flood & Cherkin, 1987; Goodman, Flexner, & Flexner, 1975; Hyman, Malenka, & Nestler, 2006; Kalivas, 2005; Kelley, 2004).

### 3.2. Mechanisms of action and related toxicity of protein synthesis inhibitors

Adding to doubts over the specificity of protein synthesis inhibitors on translation is the fact that they are all significantly toxic at levels needed to cause amnesia. Yet, the antibiotics puromycin, anisomycin, emetine, cycloheximide and acetoxycycloheximide continue to be widely used to inhibit translation *in vivo* (Davis & Squire, 1984; Stork & Welzl, 1999; Uphouse, MacInnes, & Schlesinger, 1974). The following section summarizes some of the issues surrounding mechanisms of translation inhibition and toxicity. Puromycin acts by incorporating into the growing peptide chain causing premature release from the ribosomal complex and the production of abnormal peptidyl-puromycin fragments (Flexner & Flexner, 1968b; Nathans, 1964). Side effects include hippocampal seizures, swelling of mitochondria, and disaggregation of ribosomes (Flood, Rosenzweig, Bennett, & Orme, 1973). Emetine complexes with 80S ribosomal subunits impairing amino acid-tRNA binding. Cycloheximide and acetoxycycloheximide AXM inhibit chain initiation as well as chain elongation by interacting with 60S ribosomal subunits but also impair DNA and RNA synthesis (Gale,

Cundliffe, Reynolds, Richmond, & Waring, 1981). Anisomycin, presumably having fewer side effects than other inhibitors (Flood, Bennett, Orme, & Rosenzweig, 1975; Flood et al., 1973; Squire & Barondes, 1974), interferes with protein synthesis by inhibiting chain elongation (Pestka, 1971; Vazquez, 1979). All protein synthesis inhibitors cause visible symptoms of distress (e.g., piloerection, lethargy, diarrhea, and gustatory aversions) when administered systemically in rodents (Davis & Squire, 1984; Squire, Emanuel, Davis, & Deutsch, 1975). Historically, the toxicity of protein synthesis inhibitors has been deemed acceptable as long as the animal's physical ability to learn or perform is not compromised. Post-training administration of protein synthesis inhibitors followed by behavioral testing after the effects of the drug have dissipated is a method commonly used to avoid the confounding effects of drug toxicity on memory readouts (but see Hernandez & Kelley, 2004). It should also be noted that the RNA synthesis inhibitor actinomycin D acts by binding DNA at the transcription initiation complex preventing elongation by RNA polymerase (Sobell, 1985). However, use of this drug has been plagued by the fact that it has been shown to cause necrosis and electrical abnormalities in the brain as well as behavioral signs of toxicity that result in death when delivered systemically (Barondes & Jarvik, 1964; Nakajima, 1969; Wetzel, Ott, & Matthies, 1976). The less toxic mRNA synthesis inhibitor 5,6-dichloro-1- $\beta$ -D-ribofuranosylbenzimidazole (DRB) acts by inhibiting RNA polymerase II and has been successfully used as an alternative to actinomycin D in more recent studies to impair memory (Apergis-Schoute, Debiec, Doyere, LeDoux, & Schafe, 2005; Huang, Martin, & Kandel, 2000; Nguyen, Abel, & Kandel, 1994).

### 3.3. MAPK activation and apoptosis: Protein synthesis inhibitor induced death or survival?

There is some concern that protein synthesis inhibitors might impair memory via apoptotic cell death. Indeed, protein synthesis inhibitors, especially anisomycin, have long been used as apoptotic agents in cell culture (Shifrin & Anderson, 1999). Anisomycin is thought to cause apoptosis through ribotoxicity or through the activation of MAP kinases (the role of MAPK in learning and memory is discussed in more detail below) (Iordanov et al., 1997). MAPK transduces extracellular signals from tyrosinekinase receptors and guanine nucleotide-binding regulatory proteins (G-protein) coupled receptors to cytoplasmic and nuclear effectors (Chang & Karin, 2001; Schaeffer & Weber, 1999). Three related MAPKs have been identified in neurons: the extracellular signal regulated kinase (ERK), the c-Jun terminal kinase (JNK), and p38 (Obata et al., 2004). ERK signaling cascades mediate cell development, survival and plasticity (Seger & Krebs, 1995; Sweatt, 2001), whereas p38 and JNK, both of which are activated by anisomycin (Eriksson, Taskinen, & Leppa, 2007), respond to cellular stress promoting cell death (Coffey, Hongisto, Dickens, Davis, & Courtney, 2000; Ip & Davis, 1998; Kyriakis & Avruch, 1996; Kyriakis, Woodgett, & Avruch, 1995). However, the exact role of these kinases in cellular survival and death is complex and varies according to cell type, culture conditions, and state of differentiation. For example, JNK activation in differentiated cells causes apoptosis, whereas in undifferentiated cells it leads to differentiation with no adverse effect on cell viability (Heasley et al., 1996; Le-Niculescu et al., 1999; Leppa, Eriksson, Saffrich, Ansorge, & Bohmann, 2001; Xia, Dickens, Raingeaud, Davis, & Greenberg, 1995). With regard to p38, studies suggest that inhibition of p38 signaling can be both lethal and protective (Harada & Sugimoto, 1999; Hong, Qian, Zhao, Bazy-Asaad, & Xia, 2007; Horstmann, Kahle, & Borasio, 1998; Kharlamov et al., 1995; Maroney et al., 1998; Schaeffer & Weber, 1999; Zheng & Zuo, 2004). Xia and colleagues reported that in PC12 cells that a balance exists between ERK and JNK-p38 in determining whether the cell survives or undergoes apoptosis (Xia et al., 1995). Thus, it seems unclear, at best, how plasticity-related increases in ERK activity *in vivo* might interact with anisomycin, for example, with regard to the balance of ERK/JNK-p38 signaling and apoptosis. However, two pieces of *in vivo* evidence exist suggesting that apoptosis is not a major contributor to amnesia induced by protein synthesis inhibitors. First, Lopez-Mascaraque and Price demonstrated in rat cerebral cortex

that anisomycin or emetine actually *prevented* apoptosis in cortical cells at doses used to cause experimental amnesia (Lopez-Mascaraque & Price, 1997). Second, cycloheximide, anisomycin, and emetine have been shown to be effective neuroprotective agents when coadministered with drugs of abuse such as methamphetamine in rats (Finnegan & Karler, 1992) or against hydrogen peroxide induced apoptosis (Gardner et al., 1997). Thus, the effects of protein synthesis inhibitors *in vitro* may have little to do with their actual effects on the brain.

### 3.4. Failure to impair long-term memory by protein synthesis inhibition

The resistance of some forms of memory to the effects of protein synthesis inhibitors (Dunn, Gray, & Iuvone, 1977; Flexner & Flexner, 1966; Flexner et al., 1966; Rainbow, Hoffman, & Flexner, 1980) has contributed to suspicions that consolidation does not depend on protein synthesis. Upon closer inspection, however, many of these studies were actually designed to better define the boundary conditions (e.g., training parameters, dosing schedules) in which protein synthesis inhibitors produce amnesia (Cherkin, 1969). For example, Rainbow and colleagues, reported that cycloheximide had no effect when administered to mice 2 h prior to training. However, at 30 min prior to training, when protein synthesis inhibition was significantly greater at the time of training, memory for the same tasks was significantly impaired. Additionally, Flexner's group failed to produce amnesia consistently in mice after the administration of acetoxycycloheximide 20–24 h *post-training*, long after the bulk of synaptic consolidation is thought to occur. Yet, this study is still cited as evidence that protein synthesis is not required for consolidation. Finally, in the study by Dunn and colleagues (Dunn et al., 1977), emetine and pactamycin failed to impair memory for a passive avoidance task in mice. A closer inspection of their results reveals that the doses of emetine and pactamycin used resulted in ~50% inhibition of cerebral protein synthesis—far less than typically needed to produce amnesia. Anisomycin and cycloheximide were also tested in this study and were found to produce marked disruptions in memory for the task.

It has been proposed that extinction in mice (Lattal & Abel, 2001), olfactory memories in rats and honeybees (Staubli, Faraday, & Lynch, 1985; Wittstock, Kaatz, & Menzel, 1993), color discrimination in honeybees (Wittstock & Menzel, 1994), and some forms of long-term potentiation (a leading cellular model of memory) (Kurotani, Higashi, Inokawa, & Toyama, 1996) are not affected by protein synthesis inhibitors. However, extinction in other preparations is sensitive to protein synthesis inhibitors (Berman & Dudai, 2001; Eisenberg, Kobil, Berman, & Dudai, 2003; Quirk, 2004). Interestingly, spontaneous recovery of an extinguished appetitive olfactory memory in honeybees was blocked using emetine (Stollhoff, Menzel, & Eisenhardt, 2005) demonstrating that some form of olfactory memory in the honeybee appears to be sensitive to protein synthesis inhibitors. As Lattal and Abel (2001) point out, differences in the nature of the task, procedural details (e.g., strength of the conditioned stimulus), and molecular differences by which extinction memories might be acquired could account for these contradictory findings.

### 3.5. Much ado about nothing?

At first glance, the issue seems simple enough: inhibitors of protein synthesis have a range of unintended side effects that could account for experimentally induced amnesia. Why, then, has this debate never been resolved? As we attempted to convey above, the field has been permeated with conflicting results, conclusions based on negative or misinterpreted results, and a reliance on *in vitro* studies using various undifferentiated, non-neuronal cell lines. Thus, it is important to realize that a simple demonstration of the existence of side effects of translation inhibitors without an understanding of the *totality* of those effects does not negate the preponderance of evidence in favor of a role for protein synthesis in memory consolidation.



However, could it be that critics of the role of *de novo* protein synthesis in memory formation are right but for the wrong reasons?

#### 4. Synaptic plasticity, long-term memory and protein synthesis: Progress amid the debate

Before we entertain this question, it is important to describe some of the advances the field has achieved since the 1960s and 1970s. The early pharmacological experiments with inhibitors of protein synthesis captured the interest of biochemists and molecular and cellular biologists, providing the inspiration to tackle complex behavioral phenomena including learning and memory. Indeed, a wide range of significant insights into memory storage have since been gained (Kandel, 2001). These cellular and molecular studies have defined the role of transcription and translation in synaptic plasticity and memory storage.

##### 4.1. The discovery of LTP

Some 20 years after Hebb had postulated such a phenomenon, Bliss and Lømo found that a high frequency train of action potentials resulting from stimulation of the perforant path in the rabbit hippocampus led to a long-term potentiation (LTP) of synaptic transmission in the dentate gyrus (Bliss & Lømo, 1973). That activity-dependent synaptic plasticity could be induced for long periods of time by relatively physiological stimuli in a structure known to be involved in memory was extremely significant (Acsady & Kali, 2007; Eccles, 1979; Kullmann & Lamsa, 2007; O'Keefe & Nadel, 1978; Squire, Knowlton, & Musen, 1993). The significance was further realized upon development of the *in vitro* hippocampal slice preparation (Alger & Teyler, 1976; Lynch, Dunwiddie, & Gribkoff, 1977; Schwartzkroin & Wester, 1975). The slice preparation would revolutionize how years of theoretical supposition regarding the mechanisms of associative memory formation would be explored. For example, the slice preparation made it possible to directly measure and image activity-dependent changes in calcium levels, record from specific neurons, receptors, and channels, and assess the effects of various pharmacological agents on a multitude of cellular processes (e.g., intracellular signaling, transcription, and translation). The key question, of course, is how these data regarding LTP in hippocampal slices relate to behavior. Importantly, a number of correlations and interactions between behavior and LTP have since been demonstrated (Barnes, 1979; Fedulov et al., 2007; Martin, Grimwood, & Morris, 2000; Sharp, McNaughton, & Barnes, 1985; Weisz, Clark, & Thompson, 1984; Whitlock, Heynen, Shuler, & Bear, 2006; Wilson, Willner, Kurz, & Nadel, 1986). Considering the many similarities between LTP and memory, it is now widely held that LTP holds the key to understanding how memories are formed on a cellular and molecular basis. Yet, it should be noted that the slice preparation obviously suffers from the lack of any sort of influence from systems processes.

##### 4.2. Role of protein synthesis in different phases of memory formation and synaptic plasticity

With both *in vitro* and *in vivo* models available to study the cellular mechanisms underlying synaptic plasticity and memory, many details of consolidation were rapidly elucidated. As alluded to in our discussion of Hebb and H.M., consolidation theory has come to embrace the idea that memories are formed during *at least* two phases (Bourtchouladze et al., 1998; DeZazzo & Tully, 1995; Dubnau & Tully, 1998; Ghirardi, Montarolo, & Kandel, 1995; Grecksch & Matthies, 1980; Matthies, 1974; Matthies, 1989). Short-term memory is produced in the first moments after acquisition and lasts minutes to hours, whereas long-term memory is formed during a second phase, lasting from hours to days or longer depending on the organism and type of memory. Correlates in LTP termed early LTP (E-LTP) and late (L-LTP) have also been identified. Short-term memory and E-LTP depend on the post-translational modification of post-synaptic proteins, whereas long-term memory and L-LTP depend on intracellular signaling and the regulation of transcription and translation (Abel & Lattal,

2001; Frey, Krug, Reymann, & Matthies, 1988; Goelet, Castellucci, Schacher, & Kandel, 1986; Huang, 1998; Krug, Lossner, & Ott, 1984; Malenka & Nicoll, 1999; Matthies et al., 1990; Nguyen & Woo, 2003; Reymann & Frey, 2007). An intermediate form of synaptic plasticity or facilitation has also been described in chicks and rats (Allweis, 1991; Frieder & Allweis, 1978; Frieder & Allweis, 1982; Gibbs & Ng, 1979; Rosenzweig, Bennett, Colombo, Lee, & Serrano, 1993), as well as in *Aplysia californica* (Ghirardi et al., 1995; Stough, Shobe, & Carew, 2006; Sutton & Carew, 2000; Sutton, Masters, Bagnall, & Carew, 2001).

Interestingly, long-term memory in *Drosophila melanogaster* can be pharmacologically and genetically divided into two types that develop in parallel (Tully, Preat, Boynton, & Del Vecchio, 1994). One form of long-term memory, anesthesia-resistant memory (ARM), is produced after massed or spaced training procedures, and is impaired in *radish* mutants (Folkers, Waddell, & Quinn, 2006). The other form of long-lasting memory is protein synthesis-dependent, occurs only after spaced training, requires the *Drosophila* homolog to cyclic adenosine monophosphate (cAMP)-response element-binding protein (CREB), *dCREB2*, and can be blocked by the protein synthesis inhibitor cycloheximide (Folkers, Drain, & Quinn, 1993; Tully et al., 1994; Yin et al., 1994). Thus, it might be possible that genetically and functionally independent forms of long-term memory also exist in other organisms but have yet to be identified.

The persistence of protein synthesis-dependent changes involved in long-term memory formation may require recurrent rounds or waves of translation of certain proteins (Bekinschtein et al., 2007). Indeed, multiple waves of transcription and translation have been observed during the establishment of long-term facilitation in *Aplysia* (Barzilai, Kennedy, Sweatt, & Kandel, 1989) and in LTP induction in the hippocampus (Abraham et al., 1993). Furthermore, there are two or more periods during which protein synthesis inhibitors exert their amnesic effects *in vivo* during the formation of various associative memories (Bourtchouladze et al., 1998; Chew, Vicario, & Nottebohm, 1996; Freeman et al., 1995; Grecksch & Matthies, 1980).

L-LTP (Apergis-Schoute et al., 2005; Frey, Frey, Schollmeier, & Krug, 1996; Nguyen et al., 1994) and long-term memory in a variety of species (Agranoff et al., 1967; Bailey, Kim, Sun, Thompson, & Helmstetter, 1999; Castellucci et al., 1986; Codish, 1971; Crow, Siddiqi, & Dash, 1997; Huang et al., 2000; Schafe et al., 2000) can also be impaired by the inhibition of RNA synthesis. In light of these data, consolidation theory has come to hold that access to the genetic program in the form of activity dependent transcription and the subsequent synthesis of new proteins in the *soma* is required for memory formation. However, as we learn more of the function of the *dendrite* in memory formation (Steward & Worley, 2002) the actual “instructive” role of transcription in the nucleus and protein synthesis in the soma needs to be put into the context of the role of dendritic protein synthesis.

#### 4.3. From the outside in: Pathways to protein synthesis

The use of protein synthesis inhibitors paved the way for molecular and genetic dissection of complex behavioral phenomenon such as learning and memory formation. Indeed, the theoretical implications of early pharmacological studies using these inhibitors, spurred the molecular and genetic investigation into learning impairments observed in the *Drosophila dunce* mutant (Dudai, Jan, Byers, Quinn, & Benzer, 1976) leading to the realization of the importance of cyclic adenosine monophosphate/protein kinase A (cAMP/PKA)-mediated signaling in learning and memory (Byers, Davis, & Kiger, 1981; Kalderon & Rubin, 1988). Similarly, the connection between protein synthesis and memory formation was applied to *Aplysia* where it was found that protein synthesis-dependent synaptic facilitation was required for sensitization of the gill and siphon withdrawal reflex (Montarolo et al., 1986). Further study revealed a critical role for the transcription factor cAMP response element binding protein

(CREB) in this form of plasticity (Dash, Hochner, & Kandel, 1990) and rapamycin sensitive protein synthesis (Casadio et al., 1999) where LTP studies largely elucidated the critical role of *N*-methyl-D-aspartate (NMDA) receptors in subsequent signaling and the stimulation of immediate early gene expression (Morgan & Curran, 1988; Worley, Cole, Saffen, & Baraban, 1990) and new protein synthesis resulting in synaptic plasticity and long-term memory (Bliss & Collingridge, 1993; Kim, DeCola, Landeira-Fernandez, & Fanselow, 1991; Morris, 1989; Nakazawa, McHugh, Wilson, & Tonegawa, 2004; Rao & Finkbeiner, 2007; Verkhatsky & Kirchhoff, 2007).

These seminal studies initiated much research focused on understanding how learning-related environmental information is transduced to affect synaptic change. It is now known that, once activated by coincident pre-synaptic release of glutamate and post-synaptic depolarization, NMDA receptors permit calcium entry into the cell triggering a range of intracellular signaling cascades some of which result in plasticity-related gene transcription and translation (Collingridge, Kehl, & McLennan, 1983; Cotman et al., 1989; Kasten, Fan, & Schulz, 2007). NMDA receptor signaling through the cAMP/PKA/CREB pathway has been extensively studied (Blokland, Schreiber, & Prickaerts, 2006; Dash, Moore, Kabori, & Runyan, 2007; Skoulakis & Grammenoudi, 2006; Wu, Zhou, & Xiong, 2007). Briefly, the influx of calcium stimulates calcium binding protein  $\text{Ca}^{2+}$ /calmodulin to increase the production of cAMP by adenylyl cyclases (Eliot, Dudai, Kandel, & Abrams, 1989). cAMP can also be synthesized by adenylyl cyclases linked to G-proteins upon the binding of non-glutamate transmitters (e.g., dopamine) and hormones to their receptors (Tang & Gilman, 1991). cAMP then activates PKA which in turn activates CREB (Impey et al., 1998; Roberson et al., 1999; Vanhoutte et al., 1999a). CREB can also be activated through calcium signaling through the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway where a number of important kinases including CaMKII, phosphatidylinositol 3-kinase (PI3-kinase), and protein kinase C (PKC) are involved in signaling from the NMDA receptor to MAPK/ERK (Davis & Laroche, 2006; Miyamoto, 2006; Soderling & Derkach, 2000; Wang & Storm, 2003). Thus, MAPK/ERK is thought to coordinate cross-talk between several different kinase signaling pathways, providing an additional level of control over CREB-mediated transcription during memory formation (Impey et al., 1998; Sweatt, 2001; Vanhoutte et al., 1999b; Wang, Fibuch, & Mao, 2007).

CREB along with its co-activator CREB binding protein (CBP) is then primed to regulate the expression of a variety of plasticity-related genes with CRE response elements in their promoter region (Bacskai et al., 1993; Hagiwara, Shimomura, Yoshida, & Imaki, 1996). A number of these genes are immediate-early genes and act as transcription factors regulating the expression of "effector" or late response genes. Ultimately, it is the protein products of effector genes that compose the structural components needed for the growth and/or stabilization of synapses needed in memory formation (Bailey, Bartsch, & Kandel, 1996; Milner, Squire, & Kandel, 1998; Steward, Wallace, Lyford, & Worley, 1998; Yin & Tully, 1996). Recent work also suggests that CREB/CBP is able to direct long-term epigenetic changes to structure of chromatin by acetylating histones and that this pattern of histone acetylation could mediate memory in this fashion by providing the transcriptional machinery access to plasticity-related genes (Korzus, Rosenfeld, & Mayford, 2004; Levenson & Sweatt, 2006; Vecsey et al., 2007). Indeed, mice with mutations in various plasticity-related genes, in particular those with altered PKA, CREB, or CBP activity, demonstrate marked disruptions in learning and memory (Abel et al., 1997; Bourtchouladze et al., 1994; Bourtchouladze et al., 2003; Brandon et al., 1995; Korzus et al., 2004; Oike et al., 1999; Wood et al., 2005; Yin et al., 1994) (but see Balschun et al., 2003).

A third pathway, involves signaling by target of rapamycin (TOR) proteins. The TORs are evolutionarily conserved protein kinases that regulate the balance between protein synthesis

and degradation. Kandel and coworkers implicated TOR signaling in long-term facilitation in *Aplysia* neurons by demonstrating that serotonin stimulated synaptic protein synthesis can be blocked with rapamycin, a potent inhibitor of TOR proteins (Casadio et al., 1999). In mammals TOR or mTOR is thought to modulate translation of mRNAs via the regulation of the phosphorylation state of several different translation effector proteins including the ribosomal S6 kinases, 4E-BPs, eIF4GI, eEF2, and eIF4B although much is unknown of these mechanisms (Carroll, Dyer, & Sossin, 2006; Raught, Gingras, & Sonenberg, 2001).

Knowledge of the intricacies of these ubiquitous signaling mechanisms (Deisseroth, Bito, & Tsien, 1996; Frank, 1994 #6638; Giovannini, 2006; Kaang, Kandel, & Grant, 1993; Lamprecht, Hazvi, & Dudai, 1997; Raught et al., 2001; Silva, Kogan, Frankland, & Kida, 1998; Warburton et al., 2005), although greatly informative on one hand, speaks little to the spatial regulation of translation within the neuron—an issue that created an interesting twist in our conception and definition of consolidation. Even so, we provide this summary not only to elaborate on consolidation theory but to demonstrate that despite the interpretational difficulties surrounding protein synthesis inhibitors great strides have been made that uphold the findings of studies using these “dirty” drugs. It could therefore be argued that molecular and genetic approaches have, in effect, negated worries over the non-specificity of protein synthesis inhibitors.

## 5. The changing role of protein synthesis in consolidation theory

The past few decades of research have shed light on activity-dependent processes that lead to changes in gene expression and the subsequent induction of protein synthesis in the soma. However, it is unclear how such changes are able to alter the strength of particular synaptic connections within a neuron. Returning to our previous question: could it be that *de novo* protein synthesis is not the main mechanism by which memories are permanently stabilized? Recent data suggests that the nucleus is not alone in having control over long-term memory formation but that local protein synthesis in the dendrites might also influence the synaptic stability required for the formation and maintenance of memory.

### 5.1. Stabilization by local protein synthesis?

The role of dendritic or “local” protein synthesis in learning and memory was first hypothesized in 1965, after Bodian demonstrated the existence of ribosomes in the dendrites of in monkey spinal cord motoneurons (Bodian, 1965). This was a significant finding in that it had been long-assumed that protein synthesis only took place in the soma. Ribosomes were subsequently found in the dendrites of neurons of dentate granule cell neurons (Steward & Levy, 1982). The incorporation of radiolabeled amino acids in proteins were subsequently detected in synaptic fractions (Rao & Steward, 1991; Torre & Steward, 1992; Weiler & Greenough, 1993) and in the dendrites of hippocampal slices with latencies too short to be the result of transcription and the subsequent transportation of newly synthesized protein from the soma to the dendrite (Feig & Lipton, 1993). Importantly, recent findings (reviewed by Sutton & Schuman, 2006) have demonstrated a role for local protein synthesis in the modulation of synaptic transmission (Kang & Schuman, 1996) offering a potential mechanism by which selective synaptic changes might be conferred to recently activated circuits. Thus, even with a wealth of data demonstrating the importance of transcription factors in the regulation of gene expression and protein synthesis during memory consolidation, we are now forced to reconsider the actual role these events play relative to those that occur at or near the synapse itself in response to learning-related stimuli.

## 5.2. Targeting by tagging

If local protein synthesis plays a significant role in memory formation it must be shown that a dialog between the nucleus and synapse exists whereby mRNAs are transported to those sites where local synthesis of proteins is needed. Indeed, specific mRNAs can be dendritically targeted (Garner, Tucker, & Matus, 1988; Martin, 2004; Martin & Zukin, 2006; Tongiorgi, Righi, & Cattaneo, 1997; Wang & Tiedge, 2004). For example, mouse CaMKII $\alpha$  mRNA is dendritically localized via targeting sequences in its 3'UTR and undergoes activity-dependent synaptic translation (Mayford, Baranes, Podsypanina, & Kandel, 1996; Richter & Lorenz, 2002; Rook, Lu, & Kosik, 2000). Mice with a mutation in the 3'UTR region of the CaMKII $\alpha$  gene display a reduction in CaMKII $\alpha$  levels in post-synaptic densities and impaired L-LTP and long-term memory for a several tasks (Miller et al., 2002). Knowledge of the complete range of functional control afforded by local translation, however, is limited by the number of known mRNAs localized at the dendrite. How the products of gene expression might be efficiently targeted and transported to only the correct subset of synapses needed for memory formation within a particular neuron remains the subject of great interest and debate. The notion of synaptic "tagging" or "capture" has been proposed to deal with such a problem (Reymann & Frey, 2007). Using LTP as a model for memory, Frey and Morris originally proposed that a temporary protein synthesis-independent tag is established at potentiated synapses that sequesters the relevant proteins needed to maintain LTP. In support of this notion, they found that weak stimulation of one set of synapses, which ordinarily leads to E-LTP, surprisingly resulted in the expression of L-LTP given the strong stimulation of a separate set of synapses terminating on the same post-synaptic neuron had just occurred (Frey & Morris, 1997; Frey & Morris, 1998).

Studies conducted by Martin and colleagues in *Aplysia* have also provided evidence of tagging or capture. In a culture system consisting of a single bifurcated sensory neuron that had formed synaptic connections with two spatially separated motor neurons, a series of five pulses of serotonin (5-HT) applied to one synapse resulted in facilitation of that synapse, whereas the potency of the other was unchanged. Importantly, this effect was transcription and CREB dependent demonstrating that the nucleus had some role in directing plasticity to a specific, previously activated synapse (Martin et al., 1997). Casadio and colleagues then demonstrated that the application of five pulses of 5-HT to the soma of the sensory neuron lead to a temporary, protein synthesis-dependent, cell wide form of facilitation that could be captured by specific synapses after administration of one additional pulse of 5-HT. Thus, *both* instructive protein synthesis mediated by the nucleus *in addition* to local protein synthesis at the synapse appear to play a role in memory storage (Casadio et al., 1999). To explain these results the tagging hypothesis proposes that gene products are dispersed throughout the cell but that they function to increase synaptic strength only in activated synapses that have recently been tagged. The identity of the tag, although still unknown, might be a single molecule, a complex of molecules, or a *process* such as activation of local translation, cytoskeletal reorganization, or retrograde signaling (Martin & Kosik, 2002). Importantly, no behavioral correlate of synaptic tagging has been demonstrated and therefore tagging remains a controversial matter.

Regardless, it seems that consolidation theory must be modified to account for the ability of dendrites to regulate protein synthesis at the level of the synapse, at least to some extent, without activity-dependent instructions originating in the nucleus. However, such a modification to consolidation theory has important implications. For example, what percentage of learning- or activity-dependent protein synthesis serves to simply replenish stocks of proteins or mRNAs at the synapse or dendrite? How "instructive" or involved is the genetic program of the neuron at the time of memory formation? To what extent is this program initiated before learning-related events occur (i.e., during the development of the neuron)? Is gene expression involved primarily in the maintenance of memory? All of these questions speak to whether memory lies



at the synapse or in the nucleus. It is clear, however, that tactics beyond the use of protein synthesis inhibitors will be required to answer these important questions.

## 6. New approaches in the investigation of protein synthesis in memory formation

The inadequacies of protein synthesis inhibitors still in common use must be overcome and new methods that target translation must be developed. Indeed, the challenge we currently face in terms understanding learning-related protein synthesis is to demonstrate that which is induced not what is needed for general housekeeping functions. Here we summarize how targeted disruption of translation through genetic and pharmacogenetic approaches has recently been employed to explore the role of protein synthesis in memory consolidation without depending on protein synthesis inhibitors. One interesting genetic approach utilized mice that lacked the protein kinase GCN2, a negative regulator of translation initiation that acts by phosphorylating eukaryotic initiation factor 2 $\alpha$  (eIF2 $\alpha$ ). It was shown that the threshold for eliciting L-LTP in hippocampal slices from these mice was lowered and spatial memory was enhanced after weak training in the Morris water maze (Costa-Mattioli et al., 2005). In a similar approach, mice were engineered that lacked the translation repressor eukaryotic initiation factor 4E-binding protein 2 (4E-BP2). Interestingly, these mice demonstrated memory enhancements and impairments depending on the task (Banko, Hou, Poulin, Sonenberg, & Klann, 2006). Thus, it is possible to disrupt translation without the use of nonspecific pharmacological agents.

In a clever experiment reported by Sonenberg and colleagues, the phenotype of mice with a mutated form of eIF2 $\alpha$  (eIF2 $\alpha^{+/S51A}$  mice) was reversed by a simple pharmacological treatment. eIF2 $\alpha^{+/S51A}$  mice, in which eIF2 $\alpha$  phosphorylation is reduced, display enhanced LTP and enhanced performance in several tests of memory, however, upon administration of a small molecule inhibitor of eIF2 $\alpha$  dephosphorylation, Sal003, L-LTP and memory were blocked (Costa-Mattioli et al., 2007). Importantly, these types of studies demonstrate not only the role of specific proteins in memory formation but also the power in combining genetically encoded methods of protein synthesis inhibition with relatively temporally precise methods of pharmacological control. Further development of these systems through the use of specific promoters to alter gene/protein function within specific regions of the brain or neuron will further enhance their utility.

Behavioral assays of pharmacological and genetic manipulations, as informative as they may be, are limited in that it is impossible to see events occurring in “real time”. Cellular imaging techniques making use of fluorescent reporters to monitor changes in translation (Aakalu, Smith, Nguyen, Jiang, & Schuman, 2001; Ashraf, McLoon, Sclarsic, & Kunes, 2006; Martin & Zukin, 2006; Reijmers, Perkins, Matsuo, & Mayford, 2007) are revealing much about how plasticity-related translation is temporally and spatially regulated within the neuron. For example, imaging techniques have been used to assess the effects of interference with the normal dendritic targeting of specific mRNAs (Miller et al., 2002) and to investigate the means by which translation of mRNA remains repressed in the absence of neuronal activity (Klann & Dever, 2004; Liu, Valencia-Sanchez, Hannon, & Parker, 2005). Additionally, real-time imaging of fluorescent reporter proteins after the induction of LTP together with pharmacological manipulations has led to the novel finding that LTP induction in the hippocampus leads not only to an increase in the rate of protein synthesis but also to an increase in the active degradation of proteins (Karpova, Mikhaylova, Thomas, Knopfel, & Behnisch, 2006). Imaging can also be used to begin to investigate which proteins are synthesized at the synapse and which are transported there and by what mechanism. In fact, these techniques could be used to image gene expression itself. To this end, identification of the set of mRNAs present in dendrites will help elucidate how protein synthesis might be controlled and the role

it plays in memory consolidation (Miyashiro, Dichter, & Eberwine, 1994; Moccia et al., 2003; Zhong, Zhang, & Bloch, 2006).

## 7. Alternatives to consolidation theory

### 7.1. Post-translational modification of synaptic proteins in memory formation

In light of interpretational difficulties regarding the effects protein synthesis inhibitors on memory (and a lack of pharmacological agents that are able to specifically inhibit protein synthesis), Routtenberg and Rekart have proposed a provocative, less “translation-centric” model of memory formation centering, instead, on the function of post-translational modifications (PTMs) to the local milieu of pre-existing synaptic proteins (Routtenberg & Rekart, 2005).

At its core, PTM theory, at least as we interpret it, proposes that activity-dependent changes in the PTM state of synaptic proteins are sufficient to control various processes including the structural remodeling of synapses, changes in synaptic strength, and intracellular signaling amongst other cellular changes needed for long-term memory formation. Further, PTM theory posits that all transcription and translation occurs solely to replenish the molecules consumed in the consolidation process, in contrast to the more instructive role of the gene expression endorsed by consolidation theory. Appropriate levels of synaptic proteins would be maintained by concentration gradients and through retrograde signaling mechanisms from the post-synaptic to pre-synaptic neurons composing a sort of synaptic “dialogue” throughout the network in which the memory trace is located. Although an in-depth critique of PTM theory lies outside the scope of this review, the reader is encouraged to reference the original article for a more complete description and to evaluate whether the mechanisms cited in support of PTM necessarily exclude those put forth by consolidation theory or whether some composite of both theories might best guide future research.

However, we do consider some preliminary work aimed at testing the validity of PTM theory as the conclusions drawn from these data embody the type interpretational difficulties highlighted in this review. As an initial test of PTM theory, Holahan and Routtenberg examined the effect of alterations to the phosphorylation state of several molecules (e.g., PKA, PKC, PKG) on long-term memory through intracerebral infusions of the broad spectrum serine/threonine inhibitor [1-(5-isoquinoline-sulphonyl)-2-methylpiperazine] (H7) (Holahan & Routtenberg, 2007). The authors predicted that if the PTM states of synaptic proteins play an enduring role in the maintenance of memory then even remote memories should be sensitive to disruption by alterations in the PTM state of critical proteins. In rats, they demonstrate that a single infusion of H7 aimed at the anterior cingulate 1 h prior to testing impaired freezing 3 weeks after contextual fear conditioning. Furthermore, in mice, they report that intra-medial prefrontal cortex infusions of H7 impaired extinction of the freezing response in a similar paradigm. The observed impairments were taken as initial evidence in support of PTM theory. However, there are plausible alternative interpretations of their results. For example, *pre-testing* infusions of H7 could have simply impaired *retrieval* of contextual fear memory in the first experiment. In the second experiment, *pre-training* infusions of H7 could have interrupted the encoding or consolidation of the extinction memory by disrupting PKA-dependent translation events. Thus, it should be reemphasized that the use of semi-selective pharmacological agents are not sufficient to isolate the function of various cellular processes, in this case, to delineate the function of PTM states of translation-related signaling kinases from protein synthesis on memory formation.

Another aspect of PTM theory questions the role of local protein synthesis because proteins produced in the soma are indistinguishable from dendritically translated proteins and that some mRNAs for plasticity-related proteins have not yet been found in dendrites. However, we feel

these are simple technical limitations and that techniques utilizing live imaging of tagged proteins may soon be able to provide a clear picture of the spatial and temporal control of protein synthesis. Routtenberg and Rekart also reject synaptic tagging as a means of directing replenishment because, because tagging does not require the type of ongoing synaptic dialog they propose is necessary to maintain the trace over long periods of time. However, recent studies by Schuman and colleagues have begun to address how a form of synaptic dialogue carried out through the frequency of spontaneous miniature excitatory postsynaptic currents (minis) might influence the ongoing organization of the synapse. They reported that inhibition of minis leads to the enhancement of dendritic protein synthesis. Additionally, blockade of the NMDA receptor component of minis rapidly increased the compensatory responsiveness to glutamate at the synapse in a protein synthesis dependent fashion (Sutton & Schuman, 2006; Sutton, Wall, Aakalu, & Schuman, 2004). The authors conclude that minis (viewed here as a component of synaptic dialogue) helped maintain synaptic stability by tonically repressing the dendritic protein-synthesis machinery. Thus, there may be an important link between minis (synaptic dialogue), the maintenance of the PTM state of synaptic proteins, local protein synthesis, and tagging.

## 7.2. Sleep to consolidate: A role for sleep-induced protein synthesis in memory

A growing body of literature suggests that changes in the electrophysiological state of the brain during sleep play an important role in the consolidation and organization of memory potentially through protein synthesis-dependent mechanisms. This notion is supported by several lines of evidence. First sleep deprivation is known to impair memory and LTP (Campbell, Guinan, & Horowitz, 2002; Davis, Harding, & Wright, 2003; Gais & Born, 2004; Graves, Heller, Pack, & Abel, 2003; Kim, Mahmoud, & Grover, 2005). Indeed, both of the major types of sleep, rapid eye movement (REM) and non-rapid eye movement (NREM) sleep, are altered by learning and each have been shown to influence performance in a variety of memory tasks (Best, Diniz Behn, Poe, & Booth, 2007; Buzsaki, 1989; Hellman & Abel, 2007; Stickgold & Walker, 2007). Electrophysiological data suggest that reactivation of specific neurons within cortico-thalamic networks after learning or other wakeful experiences serves as a rehearsal mechanism whereby NMDA receptor reactivation might mediate further strengthening of previously consolidated memory via protein synthesis-dependent mechanisms (Datta, Mavanji, Ulloor, & Patterson, 2004; Kudrimoti, Barnes, & McNaughton, 1999; Lee & Wilson, 2002; Pavlides & Winson, 1989; Wang, Hu, & Tsien, 2006; Wilson & McNaughton, 1994). Importantly, DNA microarray studies and proteomic studies have demonstrated changes in plasticity-related gene expression during sleep (Basheer, Brown, Ramesh, Begum, & McCarley, 2005; Cirelli, Faraguna, & Tononi, 2006; Cirelli, LaVaute, & Tononi, 2005; Cirelli & Tononi, 1999; Mackiewicz et al., 2007). Finally, there is ample evidence that behavioral readouts of memory are significantly enhanced by sleep (reviewed by Walker, 2005).

Thus, it may be that the electrophysiological state of the brain during sleep recapitulates the activity in neural circuits that occurred during previous bouts of wakefulness (Ji & Wilson, 2007) and in doing so might initiate signal transduction cascades similar to those initiated by the original learning experience thereby enhancing previously consolidated memory (Born, Rasch, & Gais, 2006; Ganguly-Fitzgerald, Donlea, & Shaw, 2006). Nevertheless, the functionality of sleep and the molecular mechanisms by which sleep improves memory remains largely unknown (Frank, 2006; Molter, Sato, & Yamaguchi, 2007) (but see O'Hara, Ding, Bernat, & Franken, 2007; Ribeiro et al., 2002). However, it is clear that a deeper understanding of the nature of gene expression and translation that occur during sleep is required to truly understand the nature of memory.

## 8. Final thoughts

It would be difficult to identify a scientific field that has benefited so dramatically from the contributions of such numerous and diverse methodologies as has the study of learning and memory. Approaches deriving from behavior, electrophysiology, pharmacology, genetics and molecular and cellular biology have been indispensable tools in unlocking the riddles of memory formation. Although there may not be agreement on the nature of the molecular mechanisms underlying amnesia induced by protein synthesis inhibitors, these drugs have played a pivotal role in our understanding of memory formation from the role of NMDA and other receptors in initiating intracellular kinase signaling cascades, to the ability of these cascades to direct new protein synthesis and gene expression. However, we have come to a stalemate in the debate over the precise role of protein synthesis in memory consolidation. Indeed, while protein synthesis inhibitors have been a useful tool, our questions are becoming more sophisticated where the specificity of these pharmacological tools is not.

A major theme in this review is whether protein synthesis plays an instructive or permissive role in memory consolidation. Is memory actually stored in the nucleus as a set of epigenetic marks or does the synapse/dendrite have everything it needs such that translation functions to simply replenish proteins consumed in the consolidation process? If the latter case is true, how does efficient replenishment occur? Is synaptic tagging a behaviorally relevant method of marking previous synaptic activity? Are both permissive and instructive processes at work simultaneously?

Indeed, we are at a time when definitions are being rewritten as nuances in memory processing are being elucidated. What is referred to as “consolidation” no doubt encompasses what will someday be parceled more precisely into separate processes. However, the challenges facing learning and memory research can no longer be addressed through the use of non-specific protein synthesis inhibitors and other pharmacological agents. By devising methods of altering the function of proteins that are *induced* by learning and by developing ways to image processes induced by learning (Reijmers et al., 2007), we will begin to provide answers to these questions. Yet, we must recognize that it is largely because of a handful of protein synthesis inhibitors that we are closer to answering the ultimate question: what is memory?

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