

Molecular bases of long-term memories: a question of persistence

Yadin Dudai

The most distinctive attribute of long-term memory is persistence over time. New studies have uncovered many aspects of the molecular and cellular biology of synaptic plasticity, and the acquisition and consolidation of memory, which are thought to depend on synaptic plasticity. Much less, however, is known about the molecular and cellular biology of long-term memory persistence. Recent findings in the field are construed within the conceptual framework that proposes that consolidation and persistence of long-term memories require modulation of gene expression, which can culminate in synaptic remodeling. Whether modulation of gene expression, and particularly the ensuing morphological plasticity of the synapse, is permissive, causal or sufficient for the materialization and persistence of the long-term trace is, as yet, undetermined. How persistent is persistence? Renewed interest is focused on the possibility that some long-term memories consolidate anew with retrieval, and could, under certain conditions, become transiently shaky in this period of reconsolidation.

Addresses

Department of Neurobiology, The Weizmann Institute of Science, Rehovot 76100, Israel; e-mail: yadin.dudai@weizmann.ac.il

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Abbreviations

AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor
CaMKII	calcium/calmodulin-dependent protein kinase type II
cAMP	cyclic adenosine monophosphate
CRE	cAMP-response element
CREB	CRE-binding protein
CS	conditioned stimulus
LTD	long-term depression
LTM	long-term memory
LTP	long-term potentiation
MAPKs	mitogen-activated protein kinases
NMDAR	<i>N</i> -methyl-D-aspartate receptor
PKA	protein kinase A
STM	short-term memory
US	unconditioned stimulus

Introduction

The issue of how engrams persist and retain their identity over time, despite the ephemeral nature and continuous turnover of their biological substrate, is central to the discipline of memory research. This ‘persistence problem’, which is particularly relevant to long-term memory (LTM), echoes a classical philosophical puzzle, ‘The Ship of Theseus’. The ship of the mythical Greek hero was placed on display in Athens and, with time, pieces of it were replaced one by one, until none of the original remained. Is this still the same ship? In memory research, the problem is even more tantalizing: not only are we unsure of how identity is preserved over time despite the

flux of biological material, but also we are uncertain of the identities of relevant components.

Attempts to solve the memory persistence problem require multiple levels of analysis. I focus on selected recent developments in the molecular and cellular analysis of LTM. Before proceeding, however, three terms require clarification: ‘memory’, ‘persistence’ and ‘long-term’.

Memories are experience-dependent internal representations, in other words, acquired models of the world, encoded in the spatiotemporal activity of brain circuits. Their use-dependent change is probably made possible by synaptic plasticity. This is the ‘synaptic plasticity and memory’ hypothesis: “activity-dependent synaptic plasticity is induced at appropriate synapses during memory formation, and is both necessary and sufficient for the information storage underlying the type of memory mediated by the brain area in which that plasticity is observed” [1]. Hence, in molecular and cellular neurobiology, the discussion of memory boils down to that of synaptic plasticity. However, proving the ‘synaptic plasticity and memory’ hypothesis is difficult, because it requires analysis of identified synapses in identified circuits that encode identified memories. A recent study [2•] illustrates such a type of analysis in *Aplysia*. The authors establish, using a simplified preparation that allows concomitant investigation of cellular physiology and behavior, a correlation between behavioral and synaptic plasticity. Yet, evidence that synaptic plasticity actually causes the encoding and persistence of memory *in vivo* and is furthermore sufficient for it, is still much needed in the field at large [1].

Persistence also deserves a note. It is not the active state of LTM that persists throughout the lifetime of a memory. Rather, what persists is the capacity to reactivate, or reconstruct, the original, or a similar, representation by the process of retrieval. Hence, to fully understand LTM persistence, we must also understand retrieval, because the mechanisms of decoding stored information are an indispensable part of the mechanisms that make the persistence of this information feasible. The specific discussion of retrieval, however, exceeds the scope of this review.

And what about the ‘long’ in LTM? For a practicing neurologist examining a patient, LTM could mean a few minutes. For a cognitive psychologist, it could mean years. In molecular and cellular neurobiology, long-term is, conventionally, >24 hours in behaving animals, or merely >1 hour in studies of long-term potentiation (LTP) — the dominant model of synaptic plasticity in mammals. Hence, the ‘long’ in molecular studies of LTM is far from the ‘long’ in real-life LTM. Do we expect the synaptic change to persist as long as the behavioral one, especially in view

of the above remarks concerning persistence? Over time, engrams may shift locations, and, in distributed memory systems, individual synapses may die out long before the memory they subserve. To explore this fundamental issue of the interrelationship between persistent neural plasticity and persistent memory, molecular neurobiology will have no choice but to address longer memory spans than it currently does.

Who and where?

Molecular models of learning and short-term memory (STM) consider multiple types of mechanisms. Some of these might also apply to LTM.

Modulation of transmitter release

The classical model of sensitization and classical conditioning of defensive reflexes in *Aplysia* focused on short-term plasticity in transmitter release, and considered the same mechanism to contribute to long-term plasticity. Studies of LTP have rerouted much of the attention to the postsynaptic terminal (see below). Controversy, however, exists regarding the site(s) of plasticity in LTP. Recent papers provide additional evidence for the candidate role of enhanced transmitter release in LTP [3,4]. Zakharenko *et al.* [3] used a fluorescent dye, taken up by synaptic vesicles by activity-induced endocytosis and later unloaded by subsequent presynaptic activity via exocytosis. Enhanced unloading was found up to an hour after induction of hippocampal LTP, suggesting increased neurotransmitter release. Whether this persists longer is yet unclear.

Recycling receptors

The hypothesis that synaptic facilitation is achieved by increased availability of postsynaptic receptors [5] was somewhat neglected for almost two decades, but is now central to models of LTP. Excitatory synapses in the mammalian brain release glutamate onto either ionotropic or metabotropic receptors. The principal subtypes of glutamatergic ionotropic receptors include the α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPA), which mediates ongoing excitatory transmission, and the *N*-methyl-D-aspartate receptor (NMDAR), which triggers synaptic plasticity. The number of AMPAR molecules in the postsynaptic membrane is a function of the synaptic history (i.e. how much the synapse has previously been active). This can range from negligible (silent synapse), to small (depressed synapse), to large (potentiated synapse) [6,7].

Insertion of AMPARs into the synaptic membrane involves trafficking from the Golgi apparatus [8] and exocytic mechanisms shared with transmitter release [6]. Removal of AMPARs involves dynamin-dependent endocytosis of clathrin-coated vesicles [6,9]. Activity-dependent membrane trafficking of AMPARs uses intracellular sorting mechanisms different from those used in constitutive recycling [10]. LTP [11,12], brief applications of NMDA [8] or the NMDAR coagonist glycine [12] under conditions that induce LTP, as well as increased activity of calcium/calmodulin-dependent

protein kinase type II (CaMKII) [11], were all found to be associated with AMPAR insertion. In contrast, long-term depression (LTD) [13], certain protocols of NMDAR activation [9,14] and AMPAR activation [9], are accompanied by AMPAR removal. Again, the central issue here is: how long can the change persist?

Association with other receptors, enzymes, scaffolding and cytoskeletal proteins, controls the anchoring and localization of receptors in the synaptic membrane. Recycling of AMPARs in the membrane is thus expected to involve the activity of a complex proteinaceous web [11,15,16]. In *Xenopus* oocytes expressing recombinant receptors, activation of a metabotropic glutamate receptor was recently reported to accelerate NMDAR trafficking into the membrane [17]. This raises the possibility that membrane trafficking of additional types of receptors subserves lasting plasticity or metaplasticity (see also [18]). Whether NMDARs are required at all after the stage of acquisition is disputed [19,20].

Kinase-phosphatase systems

Protein kinases are considered in models of plasticity in two contexts. First, as an information storage device: the persistent activation of CaMKII provides such an example. A recent analysis, however, was unable to demonstrate a role of persistent CaMKII activity in LTP maintenance [21]. Second, as a switch that triggers other plasticity mechanisms. A number of kinases were considered as switches in both LTP and behavioral conditioning. Only a few recent examples are mentioned here. Muller [22••] measured protein kinase A (PKA) activity induced in the antennal lobe of the honeybee by associative olfactory conditioning of proboscis extension. Only multiple-trial conditioning, which induces LTM, was associated with persistent PKA activation. Inhibition of PKA during conditioning blocked LTM but not acquisition. However, inducing PKA activation in the antennal lobe — by light-induced local release of a caged cyclic adenosine monophosphate (cAMP) compound — in combination with a single trial conditioning that normally does not result in LTM, was sufficient to induce LTM. Even in prolonged activation, the decay time of PKA activation was in the minute range only, implying that the activated PKA is an induction switch rather than an autonomous LTM device. Previous reports in *Aplysia* have shown that persistently active PKA is necessary, during the first 12 hours after training, for the maturation of long-term synaptic facilitation, a cellular analogue of long-term sensitization [23].

A role for CaMKII in LTM was recently demonstrated by Frankland *et al.* [24••] (see also [25]). These authors used mice heterozygous for the null mutation of α CaMKII. Whereas the homozygous null mutation blocked hippocampal LTP and learning and disrupted cortical plasticity, the heterozygote had impaired cortical but not hippocampal LTP, and remembered normally hippocampus-dependent tasks up to three days after training but not after 10–50 days. Some types of cortical memories, whose

acquisition is hippocampus-dependent, become independent of the hippocampus with time. This period, during which the memory becomes gradually independent of the hippocampus, is termed 'system consolidation', and should be distinguished from synaptic or local consolidation, in which memory becomes resistant to interferences over the first few hours after training [26,27]. Frankland *et al.* [24**] thus showed that α CaMKII is required, probably as a switch, for system consolidation in cortical circuits.

Additional kinases, considered in recent years to be critical for LTM, include mitogen-activated protein kinases (MAPKs) and other serine/threonine and tyrosine kinases [28–31]. Because the phosphorylation/dephosphorylation balance determines the kinase-dependent threshold for plasticity, phosphatases might erase memory. A recent study reports the inducible and reversible enhancement of LTP, and of STM and LTM in a hippocampus-dependent task, in a mouse engineered to express an inhibitor of the protein phosphatase calcineurin [32*]. Again, the phosphatase probably acts as a switch rather than a device that supports the trace over prolonged time.

Immunity to molecular turnover

The current conceptual framework postulates that use-dependent modulation of gene expression confers long-term plastic changes with immunity to the brief life span imposed on single copies of protein molecules by molecular turnover [33]. Modulation of cAMP-response element (CRE)-regulated gene expression by CRE-binding protein (CREB) appears to be a universal element in this process (reviewed in [34]; evidence for the requirement for CREB in learning is constantly accumulating in many systems, e.g. [35]). Gene expression in neurons takes place over a distributed network of translational machinery in multiple subcellular compartments [36]. Specifically, dendrites are equipped with local translation machinery [37–39], synaptic activity can regulate the translation of locally available RNA [40*], and activated synapses are transiently tagged to attract delivery of macromolecules, including possibly mRNA, from the cell body [41,42]. The mechanisms of local protein synthesis and their postulated roles in synapse-specific plasticity have been recently reviewed in this journal [43]. Suffice it to say that the data, taken together, portray a picture in which synaptic and cell-wide processes act in concert. This cooperation may provide: first, specificity in use-dependent synaptic modulation; second, balance between metabolic parsimony and synaptic autonomy in the allocation of metabolic resources; third, cell-wide distribution of modified synaptic information, which could contribute to the spatiotemporal integration of synaptic events over cellular space [27].

The zeitgeist: LTM ~ $f(\text{growth})$

How do newly synthesized gene products lead to persistence of LTM, if at all? One possibility is that they are the same types of molecules that alter synaptic efficacy in the short-term (e.g. ion channels, components of transmitter

release machinery, and receptors). A second possibility is that *de novo* protein synthesis contributes to synaptic growth, culminating in the remodeling of existing synapses or the emergence of new ones. That LTM involves synaptic growth is a tenet of the neurobiological zeitgeist. Evidence has accumulated in the past few years indicating that synaptic growth and remodeling are correlated with LTP and LTM [44–46]. However, proofs of necessity, causality and sufficiency are lacking. There is also much evidence that growth factors play a role in use-dependent plasticity [47]. A recent ultrastructural analysis in the hippocampus unveiled an increase of perforated postsynaptic density spines 30 minutes after LTP induction, and an increase in the occurrence of multiple spine boutons 1–2 hours after LTP induction [48]. An increase in the total number of multiple spine boutons in the hippocampus *in vivo* was correlated with classical trace conditioning of the eyeblink reflex in the rabbit [49**]. Trace conditioning of the reflex, in which the unconditioned stimulus (US) — an airpuff to the cornea — is delivered after the offset of the conditioned stimulus (CS) — a tone — is known to be hippocampus-dependent. Delay conditioning, in which the US onset precedes the CS offset, is not hippocampus-dependent.

A more radical growth process has been proposed to subservise learning and memory in some systems: postnatal neurogenesis. Enhancement of hippocampal neurogenesis has been shown to be correlated with learning in the rat and, furthermore, was reported to be required for it [50]. Shors *et al.* [51**] used a DNA-targeted toxin to inhibit neurogenesis in the adult hippocampus without impairing LTP or the survival of mature neurons. They then subjected the rats to either the delay or trace version of classical eyeblink conditioning, and found that blocking neurogenesis impaired trace, but not delay, conditioning. Recovery of cell production was associated with regained ability to acquire trace conditioning. To date, the generality of this observation, as well as its potential relevance to the persistence of memory, is unclear. Furthermore, it is noteworthy that the whole issue of neurogenesis in the adult mammalian brain is currently hotly debated [52,53].

A note on the suitability of experimental systems

Much of the aforementioned data have been generated in studies of hippocampal LTP, or hippocampus-dependent memory. The question should be raised whether LTP and hippocampus are the most appropriate process and location, respectively, to study LTM. LTP may have evolved to encode persistence of plastic changes in the synapse over a limited period of time only, say weeks, not more; system consolidation (see above) suggests that the role of hippocampus in at least some forms of LTM is itself limited over time. The molecular analysis of LTM in the neocortex, particularly in hippocampus-dependent phases, is not an easy task to accomplish, but might provide a more suitable avenue in the search for the mechanisms of the 'long' in LTM.

Recurrent windows of vulnerability?

A particularly exciting development in LTM research is the resurgence of interest in the possibility that some items in LTM reconsolidate each time they are retrieved. It has long been known in cognitive psychology that episodic memories are reconstructed with use. The notion was less popular in neurobiology, as most investigators tended to assume that, for any memorized item, consolidation starts and ends just once. Over the years, however, a number of studies suggested that reconsolidation could occur [54].

A report by Nader *et al.* [55**] stimulated much renewed interest in this possibility. They found that the protein synthesis inhibitor anisomycin, which blocks consolidation of fear conditioning in the amygdala, produces amnesia, if administered immediately after retrieval. This and earlier findings (reviewed in [54], but see for example [56]) raise multiple questions. Is the post-retrieval amnesia only a transient inhibition of the expression of the relevant behavior? If *bona fide* amnesia indeed ensues, does a latent 'core trace' still exist with privileged stability? And does reconsolidation recapitulate consolidation, in terms of the anatomical loci and the molecular and cellular mechanisms involved?

Using inhibitory avoidance conditioning, Taubenfeld *et al.* [57*] found that memory can be disrupted by the post-retrieval systemic injection of anisomycin. However, memory is not disrupted either by the intrahippocampal infusion of anisomycin or the transcription factor CCAAT enhancer binding protein β (C/EBP β) antisense, both of which block the consolidation of a new memory in the hippocampus. This indicates that reconsolidation is not restricted to fear conditioning in the amygdala, but also that the molecular mechanisms and circuits that subserve consolidation and reconsolidation, respectively, are not identical. Moreover, in conditioned taste aversion in the cortex, post-retrieval infusion of anisomycin into the taste cortex does not lead to amnesia; on the contrary, it blocks extinction [58*] (see also [59] for results in another system). In this study, the loci and molecular mechanisms of the initial consolidation and post-retrieval consolidation were found to overlap only partially; for example, MAPK and cholinergic modulation were required for consolidation of new, but not extinguished, behaviors [58*]. Thus, the fate of the trace after retrieval and post-retrieval interference depend on the task, context and brain region involved [54,58*].

The notion that recurrent vulnerability windows exist in LTM has remarkable practical potential, for example, the deletion of anguishing post-traumatic memories by post-retrieval intervention. Some psychotherapists, of course, might claim that this is exactly what they are trying to do already.

Conclusions and future directions

More is currently known about the molecular mechanisms that subserve acquisition and consolidation of LTM than

about those that subserve its persistence. Yet persistence is, by definition, the characteristic attribute of LTM that distinguishes it from short-lived forms of memory. To understand LTM, a better understanding of the molecular mechanisms of persistence is needed.

There is overwhelming evidence that LTM requires *de novo* protein synthesis. Subclasses of signaling pathways couple synaptic modulation to regulation of mRNA translation (e.g. [37,60]). Some of the newly synthesized proteins are known to play a role in cellular growth and remodeling processes, and indeed, in some systems, synaptic growth and remodeling can be demonstrated to correlate with long-term plasticity and LTM. We do not yet know, however, whether this synaptic remodeling fulfils a causal role in, or is sufficient for, the storage of the new information. Alternatively, it might maintain homeostasis, or increase synaptic capacity to compensate for used computational and representational space, in anticipation of future activity. A gedanken experiment would be to induce long-term plasticity in a memory circuit with identified individual synapses, wait for synaptic remodeling to take place, and then eliminate the new synapses only. Will the newly learned information be retained?

New types of experiments are clearly also needed to determine whether, in some systems (e.g. the avian brain [61]), new representational space is generated by getting rid of old neurons and producing new ones. If this is the case, how do the outdated LTMs signal to their own cellular substrate that they have become dispensable and, moreover, obstructive to the formation of new LTMs? Will we further refocus our attention of the molecular biology of LTM from the level of the individual synapse to the level of the whole cell [27]?

Understanding the code(s) of internal representation at the circuit level, especially in neocortex, might also facilitate the search for those molecules that contribute directly to the retrieval and hence the persistence of LTM. Finally, models of LTM persistence will also have to take into account the emerging possibility that persistence of at least some types of long-term engrams is relative, as they can be recurrently disrupted upon retrieval. At the end of the day, will we find a master solution to the molecular mechanisms that keep memory persistent over long periods of time? What we already know about the molecular mechanisms of acquisition and short-term memory hints that this is unlikely to be the case.

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