Published in final edited form as:

Brain Res. 2015 September 24; 1621: 73–81. doi:10.1016/j.brainres.2014.11.033.

Multiple cellular cascades participate in long-term potentiation and in hippocampus-dependent learning

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Abstract

Since its discovery by Bliss and Lomo, the phenomenon of long-term potentiation (LTP) has been extensively studied, as it was viewed as a potential cellular mechanism of learning and memory. Over the years, many signaling cascades have been implicated in its induction, consolidation and maintenance, raising questions regarding its real significance. Here, we review several of the most commonly studies signaling cascades and discuss how they converge on a common set of mechanisms likely to be involved in the maintenance of LTP. We further argue that the existence of cross-talks between these different signaling cascades can not only account for several discrepancies in the literature, but also account for the existence of different forms of LTP, which can be engaged by different types of stimulus parameters under different experimental conditions. Finally, we discuss how the understanding of the diversity of LTP mechanisms can help us understand the diversity of the types of learning and memory.

Keywords

Calpain; CamKII; PKA; Actin polymerization; PTEN

1. Introduction

The discovery of the phenomenon of long-term potentiation by Bliss and Lomo in 1973 (Bliss and Lomo, 1973) launched an avalanche of studies directed at relating changes in synaptic efficacy resulting from various patterns of electrical activity in various neuronal networks to various forms of learning and memory. Fundamental to this search were the assumptions that (i) learning episodes trigger certain patterns of neuronal activity in various locations within the brain, resulting in long-lasting modifications of synaptic contacts, and (ii) the distribution of the modified synapses forms an internal representation of the memory of the events responsible for the initial neuronal activity. Recall of the memory would then consist in the reactivation of these modified synapses and networks. As usual in science, this

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search has led to numerous findings, opened numerous paths – some of them turning out to be dead-ends, generated countless debates, resulted in one Nobel prize, but has yet to provide a comprehensive theory of learning and memory. As has been repeatedly mentioned, a major difficulty to integrate all the findings is due in part to the use of different experimental protocols by the majority of research laboratories working on this topic, and the apparent lack of reproducibility of experimental data resulting from these differences.

In the original report from Bliss and Lomo, a brief train of high frequency stimulation (100 Hz, 1 s) to the perforant path resulted in a long-lasting increase in synaptic responses elicited in the dentate gyrus granule cells (Bliss and Lomo, 1973). This finding was rapidly extended to other monosynaptic glutamatergic synapses throughout the hippocampus, and it became apparent that this phenomenon could represent a cellular mechanism for some forms of learning and memory (Lynch and Granger, 1992; Barnes, 1995; Maren and Baudry, 1995; Stevens, 1998; Lynch, 2004; Sacktor, 2008). This idea was further strengthened by the discovery that theta-burst stimulation (TBS) (trains of brief bursts of stimulation delivered at theta frequency, 5–7 Hz) also produced LTP (Larson and Lynch, 1986, also see Larson, this Issue), since the electroencephalogram theta rhythm is associated with exploration of new environment and is used to synchronize activity of networks in different brain regions (Winson, 1978). Since then, HFS and TBS have been widely used by numerous laboratories to elicit LTP and to study the cellular pathways underlying LTP at the synaptic level.

Numerous molecular/cellular pathways have been shown to be involved in this process, leading some scientists to even question the biological reality of the phenomenon (Sanes and Lichtman, 1999; Lisman et al., 2003). Thus, it has been extremely difficult to reach a consensus on the details of the events critically involved in linking activation of NMDA receptors, a widely accepted mechanism required to induce LTP, to the increase in AMPA receptors and changes in dendritic spine structure, two of the most widely accepted mechanisms involved in the long lasting maintenance of LTP (Morris et al., 1986; Matsuzaki et al., 2004). Because activation of NMDA receptors leads to a brief influx of calcium in postsynaptic structures, and is critical for LTP induction by both HFS and TBS, the search for intracellular cascades has focused on calcium-dependent enzymes, which could trigger changes in structure and function of dendritic spines. Calmodulin-dependent Protein Kinase II (CamKII) was thus found to be activated and to play a critical role in LTP induced by either HFS or TBS, and it is generally thought that it serves to both transiently phosphorylate and enhance the function of AMPA receptors and to create new slots to insert AMPA receptors in postsynaptic densities (see Lisman and Raghavachari, this Issue). Two other major pathways involving different protein kinases, cAMP-dependent protein kinase (PKA) and extracellular regulated kinase (ERK), have also been proposed to link NMDA receptor activation and extracellular signals, including the neurotrophic factor, BDNF, to long-lasting changes in synaptic function.

More than 30 years ago, we proposed that another calcium-dependent process participated in LTP, consisting in the activation of the calcium-dependent protease calpain (Lynch and Baudry, 1984). At that time, very limited information was available regarding the properties of calpains and the nature of their substrates, which could be involved in LTP, with the exception of the cytoskeletal spectrin (Siman et al., 1984). Finally, the role of protein

synthesis has been debated since the beginning of the discovery of the LTP phenomenon and there is still no general consensus regarding its role in LTP induction, consolidation and maintenance (Gold, 2008). Lynch et al. (this Issue) discuss recent findings from their laboratory, which provide a potential explanation for the wide range of experimental results obtained with protein synthesis inhibitors in LTP. We recently summarized the roles of several of the pathways mentioned above in a review dedicated to the 80th birthday of Richard Thompson (Baudry et al., 2011) (Sadly, Richard Thompson passed away during the preparation of this Special Issue of Brain Research for which he still provided a review the weeks before his death). Quite remarkably, the years since then have seen an explosion of findings regarding both the mechanisms underlying LTP and the links between the properties of LTP and those of some forms of learning and memory. What has emerged over the years is the idea that multiple intracellular cascades are activated by various patterns of electrical activity, resulting in long-lasting enhancement of synaptic transmission at glutamatergic synapses, and that different parameters of the stimulus used to induce LTP (frequency, intensity, pattern) result in the activation of different intracellular cascades. Accordingly, there are probably several forms of LTP, some of them co-existing in the same synapses and some of them occurring in different synapses and possibly brain regions. As we will discuss, the debate has now shifted into identifying the relationships between the parameters of tetanic stimulation and different forms of LTP as well as between different forms of LTP and various types of learning and memory. We will start by reviewing new findings regarding the roles of the two major calpain isoforms, calpain-1 and calpain-2, in LTP induction and consolidation, which we referred to as the calpain cascades. We will then follow by a brief review of the CaMKII cascade, as this theme is discussed extensively by John Lisman (Lisman and Raghavachari, this Issue). We will then review the role of the PKA cascade in LTP. We will then briefly discuss how all these cascades converge onto a common set of mechanisms, which thus become central nodes in the integration of multiple cascades and underlie the structural modifications responsible for the maintenance of LTP.

2. The calpain cascades

The original hypothesis we proposed in 1984 (Lynch and Baudry, 1984) posited that learning-related patterns of repetitive afferent activity cause a sufficient level of depolarization of dendritic spines to elevate local calcium concentration to a degree sufficient for calpain activation. Calpain then would degrade the cytoskeletal protein spectrin, resulting in an increase in the number of glutamate receptors in post-synaptic densities. Repetition of this process would modify the structure of dendritic spines and their embedded synapses, an event that could provide for the long-term stabilization of the increased number of receptors. As we pointed out at the time, the involvement of a protease as a critical regulatory step had several implications. Because proteases produce irreversible modifications of their substrate proteins, and in the case of calpain, functional modifications resulting from partial truncation (Murachi, 1984), their activation results in alterations of cell functions that last for the duration of the lifetime of these proteins. It was thus conceivable that calpain activation could result in several functional changes that could exhibit different time-courses depending on the half-lives of the various proteins truncated by calpain. This feature could therefore account for various stages in memory formation, as

truncation of proteins with fast turn-over would be responsible for short-term functional modifications, whereas truncation of proteins with long half-lives would produce longer lasting changes. Beyond these changes directly related to protein half-lives, we postulated that calpain activation could result in very long-lasting functional modifications of synaptic function by indirectly contributing to structural modifications that could be resistant to protein turn-over, such as changing the size or shape of dendritic spines. As we concluded in our 1984 paper “if calpain activation is triggered by modest levels of calcium and produces irreversible changes in synaptic chemistry, then its activation would seem to be both a likely event and one that should produce lasting changes in the operation of neuronal circuitries”.

Although we already knew in 1984 (Lynch and Baudry, 1984) the existence in the brain of the two major calpain isoforms, μ -calpain (aka calpain-1), and m-calpain (aka calpain-2), we did not know which calpain isoform was involved in LTP and what was(were) its target(s) beyond the initial target we identified, namely brain spectrin. While we and others found that pretreatment of hippocampal slices with a broad spectrum calpain inhibitor, such as leupeptin, prevented LTP formation (Oliver et al., 1989; Denny et al., 1990), and that chronic treatment with leupeptin prevented olfactory discrimination learning (Staubli et al., 1984), these results did not provide information on either of these issues. On the other hand, experiments using down-regulation of calpain-1 by treating cultured hippocampal slices with siRNA suggested that calpain-1 was involved in LTP, although the use of hippocampal slices prepared from calpain-1 ko mice did not confirm this finding (Grammer et al., 2005). More recently, the role of calpain in LTP received strong support from results of experiments using hippocampal slices from mice with a conditional down-regulation of calpain-4, the small subunit required by both calpain-1 and calpain-2 for functional activity (Amini et al., 2013). Two major findings produced results that completely changed our view of the roles of calpain-1 and calpain-2 in LTP. We first found that BDNF, a neurotrophic factor critically involved in synaptic plasticity and in LTP (see Leal et al., and Nelson and Alkon, this Issue), could lead to calpain-2 activation through ERK-mediated phosphorylation (Zadran et al., 2009). Furthermore, calpain-2 activation by BDNF was necessary for BDNF-mediated stimulation of mTOR-dependent regulation of local protein synthesis (Briz et al., 2013), another critical process in LTP formation. Elucidation of this mechanism revealed that calpain-2 selectively cleaved the phosphatase PTEN, a negative regulator of mTOR, thus suggesting that calpain-2 activation following LTP induction could lead to increased local protein synthesis, and thus could participate in LTP consolidation. The other finding illuminating the roles of calpains in LTP was provided by results of simple experiments applying a broad spectrum, cell-permeable calpain inhibitor, calpain inhibitor III, either before or after delivering the LTP-inducing stimulus, in this case TBS, in acute hippocampal slices. These results were quite astonishing, as treatment of slices with calpain inhibitor III before TBS reproduced the initial results, namely inhibited LTP, while treatment with calpain inhibitor III immediately after TBS enhanced the magnitude of LTP, and treatment with calpain inhibitor III one h after TBS had no effect. To solve these puzzling results required introducing another player, which had previously been linked to both calpain and to LTP. Shimizu et al. (2007) had previously reported that calpain, by degrading a Ras inhibitor called the SCN circadian oscillatory protein (SCOP), regulated ERK activation in hippocampus, a kinase with numerous links to LTP (Giovannini, 2006). In a series of

experiments, we found that rapid calpain activation following TBS resulted in SCOP degradation and ERK activation. Using double-labeling for calpain activation with an antibody against calpain-mediated spectrin breakdown product, and for SCOP degradation with an antibody against SCOP, indicated that these 2 events were taking place in the same dendritic compartments following TBS. However, the rapid SCOP degradation was followed by its rapid de novo synthesis, thereby limiting the duration of ERK activation (Wang et al., 2014). This rapid synthesis required calpain-2-mediated PTEN degradation, and mTOR activation. Finally, we obtained a previously described selective calpain-2 inhibitor, the dipeptide ketoamide, Z-Leu-Abu-CONH-CH₂-C₆H₃(3,5-(OMe)₂), which had been reported to have a Ki of 22 nM against calpain-2 vs a Ki of 2.3 μM against calpain-1 (Li et al., 1996). Interestingly, this inhibitor had no effect on LTP induction when applied before TBS, but produced the same increase in LTP magnitude starting about 10 min after TBS. Likewise, it produced a similar increase in LTP magnitude when applied 10 min after TBS (Wang et al., 2014). All these results led to a new model for LTP induction and consolidation following TBS (Fig. 1). In this model, calpain-1 is rapidly stimulated by the calcium influx generated by NMDA receptor activation, resulting in spectrin degradation, as in our initial model, and in SCOP degradation. Rapid calpain-1 activation triggers a number of different signaling pathways, including cytoskeleton reorganization as a result of RhoA degradation, and the ERK pathway through SCOP degradation. In the following minutes, calpain-2 is in turn phosphorylated and stimulated through BDNF-mediated further ERK activation; this leads to PTEN degradation, mTOR activation and stimulation of the synthesis of a number of proteins with mRNAs located in dendrites, including SCOP. Restoration of normal SCOP levels inhibits ERK, thereby terminating the induction process for LTP. In other words, this mechanism constitutes a molecular brake, which limits the extent of potentiation following a single TBS episode, and provides a plausible explanation for recently discovered timing rules for LTP. In particular, it has been reported that a second episode of TBS does not produce additional LTP unless delivered after a delay of about 1 h, at which point it doubles the magnitude of potentiation (Kramar et al., 2012). It thus appears that LTP induction and expression are followed by an extended refractory period. Additional work suggested that adult hippocampus contains a large population of spine synapses with high plasticity threshold and that these are not driven into the potentiated state by a single TBS episode (Lynch et al., 2013). Notably, the timing of the dual role of calpain in regulating SCOP metabolism matches that for the refractory period during which the high threshold synapses do not respond to a second TBS. If the two phenomena are closely related, then removing the calpain-mediated brake on LTP magnitude should occlude the enhancement of LTP produced by the second TBS. We confirmed this prediction by showing that a delayed TBS had no effect on the enhanced potentiation produced by a brief, post-TBS application of a calpain inhibitor. Thus calpain-2-mediated increase of SCOP synthesis in the minutes following LTP depresses ERK activity at high threshold synapses and thereby prevents them from transitioning into the potentiated state. In this revised LTP model, calpain-2 activation serves to restrict single trial encoding of new information to a subset of synapses and imposes a delay before the initial representation can be expanded. This mechanism could be directly related to the well-known distributed practice effect, a fundamental aspect of learning that has received little attention from neuroscientists.

Specifically, training trials spaced apart by sizable delays produce much stronger memory than does a single, massed learning session (Benjamin and Tullis, 2010).

In our new model of the calpain cascade (Fig. 1), we also incorporated other signaling pathways, which have been also demonstrated to participate in LTP induction and consolidation. We recently reviewed the mechanisms linking TBS to cytoskeletal regulation involved in LTP consolidation, and in particular the pathways connecting TBS to actin polymerization in dendritic spines (Baudry et al., 2011). Two actin-signaling pathways involve the Rho family of small GTPases, RhoA, Rac and Cdc42, which are ubiquitously implicated in the mechanisms of actin filament assembly, disassembly, or stabilization in most cells (Burridge and Wennerberg, 2004), and have been shown to be critically involved in LTP consolidation (Chen et al., 2007; Rex et al., 2009). However, while actin filaments can form *in vitro* by polymerization from actin monomers into oligomers followed by rapid elongation until a steady-state is reached when rate of depolymerization equals rate of polymerization, the process taking place inside cells is regulated by numerous interacting proteins (Pollard and Cooper, 2009; Dominguez and Holmes, 2011). In unpublished data, we found that calpain-1 and calpain-2 have also opposite effects on RhoA, thereby providing another mechanism linking calpain activation to regulation of actin filaments and dendritic spine cytoskeleton. Furthermore, it has also been recently shown that p70S6K can phosphorylate PAK (Gu et al., 2013), which would provide another link between the calpain cascade and regulation of actin polymerization. Finally, calpain-2 has also been shown to degrade the Striatal-Enriched protein phosphatase, STEP (Xu et al., 2009; Wang et al., 2013), and STEP has been involved in AMPA receptor endocytosis (Zhang et al., 2008).

Thus, the calpain cascades are linked to all the events, which have been proposed to participate in synaptic plasticity, including cytoskeletal regulation, AMPA receptor trafficking, actin polymerization and regulation of local protein synthesis.

3. The protein kinase cascades

Several protein kinases, including CamKII, PKA, and ERK, have been implicated in LTP elicited by various types of stimulation, and numerous reviews have been written to illustrate the various pathways upstream and downstream of these kinases and their links to various mechanisms involved in LTP induction or consolidation (Nguyen and Woo, 2003; Waltereit and Weller, 2003; Giovannini, 2006; Okamoto et al., 2009; Hell, 2014; Shonesy et al., 2014). While the upstream events are all linked to activation of NMDA receptors and the resulting calcium influx, the downstream events selectively activated by all the different kinases have been much more difficult to identify. Nevertheless, CamKII has been shown to be directly activated by calcium influx through the NMDA receptor/channel, and its role has been proposed to phosphorylate AMPA receptors, thereby producing the well-known postsynaptic potentiation, and to regulate insertion of additional AMPA receptors in postsynaptic densities (Fig. 2). John Lisman has also extensively discussed the idea, initially proposed by Francis Crick that CamKII represents a molecular switch, which exists in an off or on state and can be maintained in the on state for prolonged periods of time based on the molecular properties of the holoenzyme (Lisman and Raghavachari, this Issue). Another property of CamKII, which has only recently been stressed and reviewed (Okamoto et al.,

2009; Hell, 2014), is its ability to bind actin and to regulate the actin cytoskeleton. In particular, CamKII β binds to and cross-links filamentous actin; in addition it also binds to α -actinin, another protein interacting with filamentous actin, and this binding is dynamically regulated by calcium and the calcium-calmodulin complex. Moreover, CamKII β association with actin filaments facilitates bundling of actin filaments and dendritic spine enlargement in a kinase-independent manner. Thus, calcium influx through NMDA receptors is now postulated to have 2 effects on the distribution of CamKII in dendritic spines, and in the size of dendritic spines: it rapidly releases CamKII from actin filaments, facilitating its binding to the C-terminal domain of GluN2B subunits of NMDA receptors. In addition, CamKII is also involved in the regulation of stargazin association with AMPA receptors, as CamKII-mediated phosphorylation of stargazin is required for the immobilization of AMPA receptors in postsynaptic densities (Opazo et al., 2010). The rapid CamKII activation is followed by a slower redistribution of CamKII from dendritic shafts to dendritic spines and bundling of actin filaments and dendritic spine enlargement. All these results indicate that CamKII exerts multiple effects on the regulation of synaptic plasticity (Fig. 2).

The role of the cAMP-dependent protein kinase, PKA, appears to be more complicated and in particular, to depend on the pattern and intensity of electrical stimulation used to induce LTP (Nguyen and Woo, 2003). PKA phosphorylates a large number of substrates not only in the cytoplasm, but also in the nucleus. This makes the function of PKA in LTP and memory formation more complex and difficult to determine. PKA can directly phosphorylate AMPA receptors and modulate their functions (Banke et al., 2000; Carvalho et al., 2000; Shen et al., 2013) (Fig. 3). This property can therefore rapidly regulate glutamatergic transmission during the early phase of LTP. Furthermore, PKA and CamKII can both phosphorylate GluR1 subunits of AMPA receptors, thereby regulating AMPA receptor recycling. In addition, PKA also mediates CREB activation and promotes ERK nucleus translocation (Nguyen and Woo, 2003). This pathway has generally been viewed as linking extracellular signals to the synthesis of new proteins, especially of AMPA receptors, as well as to transcription regulation, thus participating in the late phase of LTP consolidation. Another pathway, which has received recent attention is the ability of PKA to phosphorylate various residues in GluN2B and GluN1 subunits of NMDA receptors, thereby regulating their gating properties or calcium permeability (Lau et al., 2009; Aman et al., 2014; Murphy et al., 2014). Finally, it is interesting to note that PKA is also involved in actin filament regulation. New actin filaments are formed by the binding of the Arp2/3 complex together with a number of proteins from the WASp/Scar/WAVE family (Machesky and Insall, 1998; Rogers et al., 2003). Filament growth is generally terminated by end-capping with a number of different capping proteins. Actin filaments also form elongated networks due to interactions with a number of actin-binding proteins (ABPs) and cross-linkers (α -actinin, fascin, and filamin) (Michelot and Drubin, 2011).

Thus, these different downstream pathways of PKA could account for the different roles postulated for PKA at various stages of LTP, i.e., induction, consolidation and maintenance., PKA has generally been proposed to participate in late LTP and memory consolidation phases. This idea is supported by the notion that PKA-mediated stimulation of protein synthesis is important for late LTP consolidation. This idea is also based on results of studies reporting no effect of PKA inhibitors on LTP induction (Otmakhova and Lisman,

1996; Abel et al., 1997). On the other hand, it has been proposed that there exists a developmental switch in the role of PKA RIIbeta in LTP, as young mice lacking this regulatory protein exhibit impaired LTP, while LTP is normal in young adult mice lacking this protein (Yang et al., 2009). Previous studies have also indicated that PKA is critical for LTP induction (Otmakhova et al., 2000; Yasuda et al., 2003; Skeberdis et al., 2006). Additionally, distinct patterns of synaptic activity can produce different forms of LTP that significantly engage different isoforms of PKA (Nguyen and Woo, 2003). These findings might also explain the various time windows during which PKA needs to be activated to participate in LTP. There is also evidence that LTP in different structures might engage different protein kinase cascades, as LTP in the dentate gyrus is inhibited by the coapplication of CamKII and PKA inhibitors, while single application of the inhibitors does not (Wu et al., 2006).

Thus, like CamKII, PKA has a variety of downstream effectors, which can have a variety of effects on the different phases of LTP as well as in different brain structures. This diversity could well account for the apparent discrepancy in the literature regarding the involvement of PKA in LTP.

4. Convergence of different signaling cascades on a common set of mechanisms

While each of the signaling cascades discussed above share a number of upstream activators (NMDA receptors, calcium, BDNF, etc.) they also converge on a common set of mechanisms (Fig. 4). Thus, they all participate in the regulation of AMPA receptor trafficking and can therefore all lead to the increased number of AMPA receptors, which is generally considered to be an important element of LTP maintenance. They also all lead to increased actin polymerization, and increased spine enlargement, another mechanism considered to be critical for LTP maintenance. Finally, they all regulate slower processes related to gene transcription, protein translation as well as protein posttranslational modifications. While the participation of these mechanisms in LTP induction, maintenance and consolidation remains controversial, they are likely to play some role in what is generally referred to as metaplasticity, i.e., the influence of various events to modify subsequent plasticity (Thiagarajan et al., 2007; Dudai, 2009; Guzman-Karlsson et al., 2014).

In addition, there exist many cross-talks between the 3 signaling cascades discussed above. Calpain has been shown to degrade various subunits of PKA both in vitro and in vivo (Liang et al., 2007), while PKA-mediated phosphorylation of calpain at serine 369–370 inhibits calpain activity (Shiraha et al., 2002; Zadrán et al., 2009). In addition, PP2A dephosphorylates calpain-2 but is itself subjected to opposite regulation by PKA and CaMKII. Calpain has also been shown to degrade CamKII (Hajimohammadreza et al., 1997), although the functional significance of this cleavage has not been extensively investigated. Considering that the activity of these 2 kinases is regulated by a variety of phosphatases, which can be targets of calpain, the number of interactions between the 3 cascades becomes very large.

The existence of these divergent/convergent mechanisms regulating synaptic plasticity suggests that depending on the conditions (experimental, genetic background, environmental, etc.) any number of different pathways could be activated, which could all lead to the formation of the same long lasting modifications of synaptic contacts, enlarged dendritic spines and increased number of AMPA receptors.

5. Relationship with learning and memory

While the relationship between LTP and learning and memory has been questioned (Shors and Matzel, 1997), there is now a broader consensus that LTP represents a cellular mechanism critically involved in the formation of certain types of memory (Lynch, 2004; Lynch et al., 2014). Accordingly, manipulations that impair LTP are expected to impair learning and memory (at least certain forms of hippocampus-dependent learning and memory), while manipulations that enhance LTP are expected to enhance learning and memory. We previously reported that intraventricular infusion of the calpain inhibitor, leupeptin, produced impairment in radial maze performance and delayed alternation learning (Staubli et al., 1984), as well as in overnight forgetting during training in the water maze (Morris et al., 1987). Since leupeptin is not a specific calpain inhibitor, these results could have been due to interactions with other proteases. However, our new findings make the testable predictions that down-regulation of μ -calpain will impair learning and memory, while down-regulation of m-calpain will facilitate learning and memory. Interestingly, down-regulation of calpain-4, the small subunit common to calpain-1 and calpain-2 was associated with impairment in the Morris water maze (Amini et al., 2013).

Elucidation of the molecular mechanisms of synaptic plasticity might shed new light on various features of learning and memory, which until now did not receive a clear explanation, such as the benefits of spaced versus massed trials for learning, or for post-training modulation of learning and memory. As indicated above, the previously reported findings that training in the novel object recognition and fear conditioning training results in SCOP degradation and ERK activation in hippocampus 5 min after training indicates that this mechanism participates in learning (Shimizu et al., 2007). The return of SCOP levels to normal 30 min after training suggests that the mechanism we identified linking TBS to calpain and ERK activation (m-calpain-mediated PTEN degradation followed by SCOP synthesis) also takes place in hippocampus under these conditions. It will be therefore extremely interesting to test the effects of down-regulating or inhibiting m-calpain to verify the role of m-calpain in the consolidation period. As extensively discussed by Jim McGaugh, a major issue in developing cognitive enhancing drugs is to distinguish the effects of drugs on learning and memory processes per se from many other processes that could enhance performance (McGaugh and Roozendaal, 2009). As a result, several laboratories have used post-training injections of a variety of drugs to determine specific effects on learning and memory processes. In many of these studies the molecular bases for the observed enhancement have not been established. Our discovery of the existence of a molecular brake that limits the magnitude of LTP during the consolidation period provides a potential molecular basis for the post-training effects of at least some compounds on learning and memory (Wang et al., 2014). We hypothesize that their effects are related to

their interactions with the molecular events involved in m-calpain activation or in the downstream cascades stimulated by m-calpain, mainly the SCOP-ERK pathway.

Finally, the existence of convergent/divergent mechanisms leading to the same synaptic modifications of synaptic efficacy suggests that it will be possible to identify new ways to treat disorders associated with learning and memory impairment by targeting elements of the pathways that are not affected by the particular disorder. This could be particularly useful for genetic developmental disorders, which have been shown to affect many of the elements of the cascades discussed above, but have also been found to be amenable to successful manipulations in adulthood (Ehninger and Silva, 2009).

Acknowledgments

This work was supported by Grant P01NS045260-01 from the NINDS (PI: Dr. C.M. Gall), and Grant R01NS057128 from the NINDS to MB. XB is also supported by funds from the Daljit and Elaine Sarkaria Chair.

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Fig. 1.

The calpain cascades. Calpain-1 is rapidly activated as a result of increased calcium concentration produced by stimulation of NMDA receptors. It cleaves RhoA, producing rapid changes in cytoskeleton, as well as SCOP, a negative regulator of ERK. ERK has been involved in the regulation of AMPA receptor trafficking. These steps are necessary for triggering the cascades leading to LTP. On the other hand, calpain-2 is activated after a few minutes as a result of BDNF-mediated ERK activation and phosphorylation. Calpain-2 cleaves PTEN, resulting in mTOR activation and stimulation of local protein synthesis. In particular, SCOP is synthesized, reestablishing ERK inhibition. mTOR also phosphorylates p70S6kinase, which phosphorylates/activates PAK, thus also participating in actin polymerization. Finally, calpain-2 cleaves the phosphatase, STEP, which has been involved in AMPA receptor trafficking. GluAs: Glutamateric AMPA receptors; GluNs: Glutamateric NMDA receptors; VDCC: voltage-dependent calcium channel; TARP: transmembrane AMPA receptor regulatory proteins; STEP: Striatal-Enriched protein phosphatase; SCOP: SCN circadian oscillatory protein; ROCK: Rho-associated coiled-coil containing protein kinase; PAK: p21activated kinase.

Fig. 2.

The CamKII cascade. CamKII is rapidly activated by calcium influx through the NMDA receptor-channel. It is released from actin filaments and binds to (1) and phosphorylates (2) the NMDA receptors, regulating their functions. It also regulates the association of stargazin/TARP to AMPA receptor and PSD-95 (3), thereby participating in the regulation of changes in AMPA receptors. Finally, CamKII is redistributed from dendritic shafts to the spines, where it can bundle and enlarge actin filaments (4). A2Ar: Adenosine 2A receptor.

Fig. 3.

The PKA cascade. PKA is activated by a variety of upstream signals, including calcium, adenosine A2 receptors, as well as catecholaminergic receptors (not shown in the diagram). It can phosphorylate AMPA receptors and regulate their functions (1), as well as phosphorylate a variety of downstream signaling cascades linked to regulation of transcription and translation (2). It can also phosphorylate NMDA receptors and regulate their functions as well (3). Finally, it can also participate in the regulation of actin filaments through phosphorylation of various actin-binding proteins (4).

Fig. 4.

Convergence of the 3 cascades on a common set of mechanisms. The three cascades discussed in the review converge on a common set of mechanisms: (1) post-translational protein modifications, (2) translational regulation and (3) regulation of gene expression. In turn these mechanisms are linked to 2 of the common events responsible for LTP: increased number of postsynaptic AMPA receptors, and increased filamentous actin, resulting in enlarged dendritic spines. Moreover, the existence of multiple cross-talks between calpains, PKA and CamKII suggest that depending on the conditions, various forms of LTP can be triggered with different features. It also suggest that genetic alterations of some elements of these cascades might be overcome by activating the intact elements of the cascades to elicit normal LTP and therefore normal learning.