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Review

Regulation of late-phase LTP and long-term memory in normal and aging hippocampus: role of secreted proteins tPA and BDNF

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Abstract

Long-lasting forms of memory are generally believed to be mediated by protein synthesis-dependent, late-phase long-term potentiation (L-LTP). L-LTP exhibits at least two distinctive characteristics compared with early phase LTP (E-LTP): synaptic growth and requirement of gene transcription and new protein synthesis. In this review, we discuss the cellular and molecular mechanisms underlying the structural and functional changes of hippocampal synapses during L-LTP, in the context of long-term memory. We describe experiments that reveal the critical role of cAMP/protein kinase A and MAP kinase pathways, and the downstream transcription factor CREB. Because transcription-dependent long-term changes are input specific, we also discuss the role of ''local protein synthesis'' and ''synaptic tagging'' mechanisms that may confer synapse specificity. We then focus on brain-derived neurotrophic factor (BDNF) and tissue plasmigonen activator (tPA), two secreted proteins that have been repeatedly implicated in L-LTP. Biochemical and molecular biology experiments indicate that the expression and secretion of both factors are enhanced by strong tetanic stimulation that induces L-LTP as well as by training in hippocampal-dependent memory tasks. Inhibition of either tPA or BDNF by gene knockout and specific inhibitors results in a significant impairments in L-LTP and long-term memory. Further work will be required to address the relationship between BDNF and tPA in various forms of synaptic plasticity, and the mechanisms by which BDNF/tPA achieves synapse-specific modulation. Finally, we discuss how the aging process affects L-LTP and long-term memory.
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Keywords: Brain-derived neurotrophic factor; Long-term memory; Synaptic plasticity

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1. Introduction

A remarkable feature of the brain is to store and recall a seemingly endless series of events and experiences in life. This capacity, known as memory, is critically dependent on the hippocampus and related limbic structures (Milner et al., 1998; Eichenbaum, 2000). Hippocampal-dependent memory can be divided into two temporally and mechanistically distinct forms: a short-term form lasting seconds to tens of minutes, and a long-term form that persists for hours, days and even weeks. A key feature that sets apart long-term from short-term memory is its dependence on new protein synthesis (Squire and Barondes, 1973). Combined cellular and behavioral studies suggest that learning and memory can be modeled by long-term potentiation (LTP), an electrophysiological measure of sustained increase in synaptic efficacy at hippocampal synapses. The memory-related changes in synaptic strength can also be divided into two temporally and mechanistically distinct phases. A single train of high frequency stimulation (tetanus, 100 Hz, 1 s), which mimics the physiological bursts of neuronal activity in mammalian hippocampus, induces an increase in synaptic efficacy that lasts for 1–2 h. This form of plasticity, called early phase LTP (E-LTP), involves modifications of pre-existing synapses as a result of rapid Ca^{2+} influx through NMDA type glutamate receptor and subsequent protein phosphorylation events (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). In contrast, repeated high-frequency stimulation at certain intervals (e.g., four trains of tetanus at every 10 min) results in long-lasting, late-phase LTP (L-LTP) that lasts as long as the recordings can be maintained (6–8 h) (Frey et al., 1988). This longer lasting form of synaptic plasticity requires activation of cAMP-dependent protein kinase (PKA) and the transcription factor CREB (Kandel, 2001). Moreover, L-LTP is dependent on new protein synthesis, similar to long-term memory. Pharmacological inhibition of protein synthesis completely blocks L-LTP (Stanton and Sarvey, 1984; Frey et al., 1988). While the expression of a number of proteins is enhanced in response to L-LTP inducing tetanic stimulation (Qian et al., 1993; Matsuo et al., 2000; Ingi et al., 2001), the specific protein synthesis product(s) responsible for the induction and maintenance of L-LTP remain to be identified.

One of the tetanus-induced proteins that has received a great deal of attention in recent years is brain-derived neurotrophic factor (BDNF), a member in the neurotrophin family of secretory proteins. While initially identified as a survival factor for peripheral neurons, BDNF has emerged as a critical secretory protein that regulates synaptic development and plasticity in the CNS (Poo, 2001; Lu, 2003). In cultured hippocampal neurons, acute application of BDNF elicits rapid changes in synaptic transmission at both excitatory and inhibitory synapses (Lessmann et al., 1994; Levine et al., 1995; Brunig et al., 2001; Baldelli et al., 2002), and these effects are mediated by either pre- or post-synaptic mechanisms (Lessmann and Heumann, 1998; Levine et al., 1998; Li et al., 1998). Gene knockout studies demonstrated that BDNF is required for hippocampal E-LTP (Korte et al., 1995; Patterson et al., 1996). It has been shown that BDNF facilitates hippocampal E-LTP by potentiating synaptic response to LTP-inducing tetanus and an enhancement of synaptic vesicle docking, possibly through changes in the levels and/or phosphorylation of synaptic proteins (Figurov et al., 1996; Gottschalk et al., 1998; Pozzo-Miller et al., 1999; Jovanovic et al., 2000). In addition to its acute effects on synaptic transmission and plasticity, BDNF also exhibits a long-term regulatory role in synapse structure and function. Long-term

application of BDNF exerts complex modulation of dendritic and axonal growth in neurons of the CNS (Cohen-Cory and Fraser, 1995; McAllister et al., 1995). BDNF is involved in activity-dependent synaptic competition and formation of ocular dominance columns in the visual cortex (Cabelli et al., 1995, 1997). Long-term regulation of synaptic transmission by BDNF has also been observed in glutamatergic and GABAergic synapses in the CNS (Rutherford et al., 1998; Vicario-Abejon et al., 1998; Huang et al., 1999; Sherwood and Lo, 1999). Taken together, the capacity of BDNF to regulate the structure and function of hippocampal synapses makes BDNF an attractive candidate protein mediating L-LTP.

Another tetanus-induced protein known to be involved in L-LTP is the extracellular serine protease tissue-plasminogen activator (tPA). tPA is an extracellular serine protease, which is widely expressed in the CNS including hippocampus (Qian et al., 1993; Salles and Strickland, 2002). Induction of L-LTP enhances the transcription of tPA in the hippocampus (Qian et al., 1993). tPA can be secreted from neuronal growth cone and axonal terminals (Krystosek and Seeds, 1981), and neuronal membrane depolarization also induces secretion of tPA into the extracellular space in the hippocampus in a Ca^{2+}-dependent manner (Gualandris et al., 1996). Activation of cAMP/PKA pathway also induces the secretion of tPA (Baranes et al., 1998). In hippocampal cultures, tPA acts extracellularly to stimulate the elongation of the axons of granule neurons, and induces the formation of functionally competent glutamatergic synapses consisting of an active, pre-synaptic secretory component and a post-synaptic recognition component enriched with glutamate receptors (Baranes et al., 1998). The main enzymatic actions of tPA are to convert inactive precursor plasminogen to the active protease plasmin (Plow et al., 1995); and to degrade certain components in extracellular matrix (Chen and Strickland, 1997; Wu et al., 2000). More recently, tPA has been demonstrated to be involved in activity-related formation of perforated synapses in the hippocampus (Neuhoff et al., 1999). Thus, tPA could also serve as a potential mediator of L-LTP.

In this review, we will discuss the cellular and molecular mechanisms underlying L-LTP in the context of long-term memory. We will also describe how aging process affects L-LTP and long-term memory. In particular, we will focus on two secreted proteins BDNF and tPA, and discuss in detail the genetic, biochemical and physiological evidence supporting their role in L-LTP, and the possible underlying mechanisms.

2. Molecular mechanisms of L-LTP and long-term memory

LTP has been adopted as a cellular model for the study of learning and memory since its discovery in the early 1970s. After three decades of intensive research, we have come much closer in understanding the cellular and molecular mechanisms underlying LTP. In particular, we have known a great deal about the mechanisms for the induction of E-LTP (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). In brief, the induction process involves the activation of NMDA receptors, a subclass of glutamate receptors. High frequency stimulation (HFS) or pairing of pre-synaptic stimulation with post-synaptic depolarization removes the voltage-dependent magnesium block of NMDA receptors. This results in a rapid rise of intracellular Ca^{2+} ($[Ca^{2+}]_i$) due to Ca^{2+} influx through NMDA receptor channels on dendritic spines of the post-synaptic neurons. According to the latest

model (Liu et al., 2004), the HFS activates NR2A-containing NMDA receptor complex and subsequent activation of Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII), leading to LTP. This is very different from low frequency stimulation (LFS), which preferentially activates NR2B-containing NMDA receptor complex and activation of the phosphotase calcineurin, resulting in long-term depression (LTD) (Mulkey et al., 1993, 1994; Liu et al., 2004). How LTP is expressed after induction is less well established. Significant progress has been made in recent years supporting a model in which a rapid rise of $[Ca^{2+}]_i$, and subsequent activation of CaMKII, triggers the insertion of GluR1-containing AMPA receptor into the post-synaptic membranes on the spines (Malinow and Malenka, 2002). Over time, GluR2/GluR3 receptor comes to replace GluR1/GluR2 receptor through a recycling process, and this process occurs preferentially at synapses that already have the AMPA receptors (Shi et al., 2001). There is also evidence that pre-synaptic glutamate release is enhanced during LTP. Much less is known about the L-LTP. In particular, the cellular and molecular mechanisms involved in the conversion of E-LTP to L-LTP have only recently begun to emerge. Recent studies have addressed this cellular consolidation process and have highlighted the importance of two highly conserved signaling cascades: PKA and MAPK. Both these signaling transduction cascades can initiate gene transcription and translation of new proteins that are necessary to maintain long-lasting changes at synapses.

Substantial experimental evidence suggests that cAMP/PKA is a key signaling pathway for L-LTP. Ca^{2+} influx induced by tetanic stimulation also activates Ca^{2+} stimulated adenylyl cyclase (AC), leading to an increase in intracellular cAMP concentration and subsequent activation of PKA. It has been shown that L-LTP induced by multiple tetani is blocked by PKA inhibitors in rat hippocampal slices (Huang et al., 1995; Nguyen and Kandel, 1996). Conversely, application of adenylyl cyclase activator forskolin induces a form of long-lasting synaptic potentiation that is also sensitive to protein synthesis inhibitors (Nguyen et al., 1994; Nguyen and Kandel, 1996). The forskolin induced synaptic potentiation occludes L-LTP induced by multiple tetanus, suggesting that these two forms of plasticity share similar underlying mechanisms (Huang and Kandel, 1994). Genetic studies indicate that AC1 and AC8 double knockout mice do not exhibit L-LTP and long-term memory (Wong et al., 1999). Conversely, overexpression of AC1 enhanced recognition memory and LTP (Wang et al., 2004). In transgenic mice that express R(AB) (inhibitory form of regulatory subunit of PKA), both PKA activity and L-LTP are decreased significantly, while E-LTP is intact (Abel et al., 1997). These are paralleled by behavioral deficits in long-term, but not short-term, memory for contextual fear conditioning. Taken together, the results suggest that PKA plays a critical role in L-LTP as well as long-term memory.

There is also strong evidence that activation of mitogen-associated protein kinase (MAPK), also known as extracellular signal-related protein kinase (ERK), is necessary for L-LTP and long-term memory. In the dentate gyrus, application of LTP-inducing stimulation leads to a rapid phosphorylation and nuclear translocation of MAPK (English and Sweatt, 1996; Davis et al., 2000a). An increase in intracellular cAMP also activates MAPK (Vossler et al., 1997; Impey et al., 1998). Treatment of hippocampal slices with the MAPK kinase inhibitor U0126 or PD98059 before and during the application of L-LTP inducing tetanus markedly reduces the magnitude of L-LTP, suggesting that MAPK

activity is required for the induction of L-LTP (English and Sweatt, 1997; Impey et al., 1998; Patterson et al., 2001; Rosenblum et al., 2002). Application of PD98059 two min after the tetanus also attenuates the L-LTP (Rosenblum et al., 2002). Thus, MAPK also appears to be important for the maintenance of L-LTP. Additionally, MAPK has been shown to be required for long-term memory (Adams and Sweatt, 2002). Inhibition of MAPK impairs long-term (spatial and fear conditioning), but not short-term, memory (Atkins et al., 1998; Blum et al., 1999; Schafe et al., 1999). Expression of a dominant-negative form of MAPK kinase in the postnatal forebrain selectively suppresses the formation of long-term memory (Kelleher et al., 2004).

Both PKA and MAPK can phosphorylate and activate CREB, a transcription factor implicated in long-term memory in Drosophila and Aplysia as well as mice and rats (Yin and Tully, 1996; Silva et al., 1998). CREB phosphorylation has been shown in hippocampal CA1 neurons after LTP-inducing stimuli (Bito et al., 1996; Deisseroth et al., 1996; Matsushita et al., 2001). Mice with a targeted disruption of the alpha and delta isoforms of CREB are deficient in L-LTP as well as fear conditioning and water maze long-term-memory tasks, but show normal short-term memory (Bourtchuladze et al., 1994; Taubenfeld et al., 1999). Similarly, intrahippocampal injection of antisense oligodeoxynucleotides against CREB mRNA results in an impairment in long-term spatial memory (Guzowski and McGaugh, 1997). Expression of the constitutively active CREB in hippocampal CA1 neurons lowers the threshold of L-LTP induction such that an E-LTP-inducing stimulus could now induce L-LTP (Barco et al., 2002). Furthermore, application of PKA inhibitors or antisense oligodeoxynucleotides against CREB interferes dendritic spine formation, as well as the responses of CREB and CREB binding protein (Murphy and Segal, 1997). Taken together, CREB activation appears to be a critical step in the signaling cascade that initiates protein synthesis and consequently leads to long-term morphological modifications underlying L-LTP and long-term memory.

The maintenance of L-LTP, accompanied by long-term changes in synaptic structure and function, requires gene transcription that occurs in the nucleus located in cell body of neurons. On the other hand, the synaptic modification involved in L-LTP is largely confined to the tetanized synapses. A fundamental question in the L-LTP field is how the newly synthesized proteins are specifically targeted to, or captured by, the tetanized synapses without affecting nearby un-tetanized synapses. Are the gene products produced in the cell body and transported specifically to, or are they produced locally at, the tetanized synapses? If the proteins are synthesized in soma, how could these newly synthesized proteins travel to the dendrites and be selectively targeted/captured to a few tetanized synapses out of tens of thousands of synapses on the dendritic tree?

The idea that a ‘‘synaptic tag’’ is generated at the activated synapses to ‘‘capture’’ plasticity-related proteins and to stabilize temporary synaptic changes was first put forwarded by Frey and Morris (Frey and Morris, 1997). In this hypothesis, strong tetanus induces proteins synthesis in soma, which produces ‘‘plasticity factors’’ that are transported to dendrites. These factors could only be captured by the tags which are previously produced by tetanic stimulation at the activated synapses. Weak tetanus (e.g. one train of 100 Hz, 1 s stimulation) is sufficient to generate a tag, but the tag is generally short-lived (only a few hours, if not bound to the plasticity factor). However, the nature of the tag is uncertain. It has been suggested that the synaptic tag could be the morphological

change of synaptic spine-neck. Widening spine-neck at the tetanized synapses would allow the newly synthesized macromolecules to flow in, but the narrow spine-neck at the non-tetanized spines would restrict plasticity factors away from entering. It is also possible that the 'tag' is an E-LTP associated protein kinase undergoing phosphorylation. This kinase contributes to temporal synaptic potentiation on the one hand, and is responsible for sequestering proteins on the other hand (Frey and Morris, 1998). Recent experiments by Barco et al. (2002) suggest a role of PKA in synaptic tagging. In mice over-expressing the constitutively active CREB, one-train tetanus, which generates the tag, was sufficient to induce L-LTP. Inhibition of PKA before and during the tetanic stimulation blocked the L-LTP in the CREB transgenic mice, suggesting that PKA is required for tagging the synapse. However, PKA is not necessary for the maintenance of L-LTP once the gene products have been captured, since application of PKA inhibitor 30–60 min after LTP induction no longer affected L-LTP. They have also provided evidence that the CRE-driven gene products may be the plasticity factors for synaptic capturing.

An alternative strategy to ensure the synapse-specific nature of the protein synthesis-dependent L-LTP is ''local protein synthesis'' at the active synapses. Several lines of experiments support this idea. First, mRNAs, polyribosomes, and protein translation machinery have been found in the dendritic spines of hippocampal neurons (Steward and Levy, 1982; Kleiman et al., 1990; Steward and Schuman, 2001; Tang et al., 2002). For example, Arc mRNA is transported into dendrites after episodes of neuronal activation, and this newly synthesized Arc protein is localized selectively at synapses that have experienced particular patterns of activity (Steward and Worley, 2002). Second, neuronal activity has been shown to stimulate protein synthesis in the dendrites of hippocampal neurons (Ouyang et al., 1997, 1999; Steward and Halpain, 1999; Ostroff et al., 2002). The best example is the rapid, tetanus-induced local synthesis of CaMKII in pyramidal neurons in the hippocampus. Genetic deletion of the 3′UTR of alpha-CaMKII mRNA which prevented dendritic delivery of the mRNA resulted in a reduction of locally synthesized alpha-CaMKII protein in dendrites. These transgenic mice exhibit deficits in L-LTP as well as memory consolidation (Miller et al., 2002). Third and most importantly, inhibition of local, dendritic protein synthesis blocked L-LTP (Bradshaw et al., 2003). When the protein synthesis inhibitor emetine is applied locally to the apical dendrites of CA1 pyramidal neurons, L-LTP is impaired at apical but not at basal dendrites. Conversely, when emetine is applied locally to basal dendrites, L-LTP is impaired only at basal dendrites. Thus, local and activity-dependent protein synthesis contributes, at least in part, to the synapse-specific modulation occurring in L-LTP. Although evidence supporting ''synaptic tag'' and ''local protein synthesis'' is both relatively limited, studies in this area have provided new insights in explaining the ''input specific'' characteristic of L-LTP and contribute significantly to the understanding of the mechanism of long-lasting synaptic plasticity as well as long-term memory.

3. Regulation of L-LTP and long-term memory by BDNF

As discussed above, genes induced by L-LTP stimuli are considered as candidates that mediate the long-term synaptic plasticity. Thus, the first clue that BDNF may be involved

in long-term synaptic plasticity came from the demonstration that the expression of BDNF mRNA is enhanced by the LTP-inducing tetanic stimulation in CA1 (Patterson et al., 1992) and dentate gyrus (Castren et al., 1993; Dragunow et al., 1993) (see Lu, 2003, for review). The fact that the activity-dependent BDNF transcription requires CREB, a cAMP-dependent transcription factor implicated in long-term plasticity and memory in many species, further supports the notion that BDNF is important for the process (Shieh et al., 1998; Tao et al., 1998). These results have inspired the efforts to correlate BDNF mRNA expression with learning and memory behaviors. For examples, radial arm maze training in rats for spatial learning and memory results in a significant increase in the BDNF mRNA expression in the hippocampus (Mizuno et al., 2000, 2003). Inhibitory avoidance training is associated with a rapid increase in BDNF mRNA expression in dentate gyrus of the hippocampus (Ma et al., 1998; Alonso et al., 2002b), and the level of BDNF mRNA is also elevated during hippocampus-dependent contextual learning (Hall et al., 2000). Furthermore, exposure of rats to Morris water maze and enriched environment rapidly and selectively induce BDNF expression (Falkenberg et al., 1992).

Another important finding supporting the role of BDNF in hippocampal LTP is that unlike other neurotrophins, the secretion of BDNF is activity-dependent (Lu, 2003). Murphy and colleagues showed that BDNF protein is sorted into the regulated pathway, and its secretion in hippocampal neurons could be induced by neuronal depolarization with high concentration of K^+ (Mowla et al., 1999; Farhadi et al., 2000). Using BDNF coupled to green fluorescent protein (BDNF–GFP), Hartmann et al. have shown that high-frequency activation of glutamatergic synapses induced the secretion of BDNF–GFP from synaptically localized secretory granules and extrasynaptic dendritic vesicle clusters (Hartmann et al., 2001). Notably, the most effective stimulus to induce BDNF secretion is the LTP-inducing tetanic stimulation (Balkowiec and Katz, 2000; Lever et al., 2001; Gartner and Staiger, 2002). Activity-dependent secretion of BDNF may modulate pre-synaptic transmitter release or post-synaptic glutamate receptors, or both, and thereby regulating hippocampal LTP.

Direct evidence for BDNF regulation of hippocampal E-LTP comes from studies in which BDNF signaling is inhibited, either by BDNF gene knockout, BDNF antisense oligonucleotides, or BDNF scavengers or antibodies. Two independent lines of BDNF knockout (−/−) mice exhibit severe impairment in LTP in hippocampal CA1 slices (Korte et al., 1995; Patterson et al., 1996). Interestingly, BDNF heterozygous (+/−) mice show similar degree of impairment as the BDNF −/− mice, suggesting that a critical level of BDNF may be required in hippocampus for LTP induction and/or maintenance. This impairment is likely caused by the deletion of BDNF gene rather than the cumulative developmental abnormalities, since the E-LTP deficits are rescued by the acute treatment on mutant slices with either exogenous recombinant BDNF (Patterson et al., 1996) or virus-mediated BDNF gene transfer (Korte et al., 1995). Reducing BDNF level by antisense oligonucleotides injected into adult hippocampus in vivo (Ma et al., 1998) or scavenging endogenous BDNF with TrkB–IgG fusion protein in vitro (Figurov et al., 1996) have been shown to reduce the magnitude of E-LTP. Substantial evidence indicates that BDNF acutely facilitates E-LTP by potentiating synaptic responses to tetanic stimulation and enhancing the synaptic vesicle docking, possibly through changes in the levels and/or phosphorylation of synaptic proteins (Gottschalk et al., 1999; Pozzo-Miller et al., 1999;

Jovanovic et al., 2000; Xu et al., 2000). Post-synaptic effects of BDNF on dentate E-LTP in slices and on NMDA receptors in cultured hippocampal neurons have also been reported (Levine et al., 1998; Kovalchuk et al., 2002). Other than preventing E-LTP induction, BDNF scavenger proteins or BDNF antibodies could abolish the already established E-LTP when applied shortly after LTP-inducing tetanic stimulation (Kang et al., 1997; Chen et al., 1999).

The fact that BDNF mRNA increases 2–4 h after the application of L-LTP-inducing stimuli raises the possibility that BDNF is also involved in late-phase LTP, since this time course correlates very well with the expression of L-LTP. Korte et al. observed that L-LTP is impaired in both BDNF (−/−) and (+/−) mice, and in slices treated with TrkB blocking antibody (Korte et al., 1995). Further studies using genetic (BDNF (+/−) and TrkB conditional knockout mice) and pharmacological approaches (BDNF antibody and TrkB–IgG) have confirmed this finding (Chen et al., 1999; Patterson et al., 2001). It appears that the effect of BDNF may be influenced by the protocols used to induce L-LTP. BDNF knockout or TrkB antibody selectively impairs L-LTP induced by theta-burst stimulation (TBS) or application of forskolin, but not by four-train tetanus (Kang et al., 1997; Patterson et al., 2001). L-LTP induced by three-train tetanus, however, was impaired in slices treated with TrkB antibody, TrkB–IgG, or TrkB conditional knockouts (Kang et al., 1997; Chen et al., 1999; Minichiello et al., 2002). It is possible that strong stimulation such as four-train tetanus may induce higher level of endogenous BDNF or some other factors that could compensate the L-LTP deficits. Another interesting finding is that BDNF may be required at different time windows of L-LTP. Certain forms of LTP could be suppressed only when BDNF antibodies are applied before and during the tetanic stimulation, but not after (Korte et al., 1995; Chen et al., 1999). Patterson et al. (2001) have demonstrated that the BDNF scavenger TrkB–IgG blocked L-LTP when applied before tetanic stimulation. However, another group has shown the impairment of L-LTP when TrkB–IgG was applied only 30–60 min after tetanus (Kang et al., 1997). The discrepancies may be due to different experimental conditions such as different stimulation protocols and different durations of drugs perfusion. Nevertheless, all the experiments addressing the functional time window of BDNF in L-LTP thus far have examined this issue indirectly by the application of TrkB antibodies or scavenger proteins. A direct approach could be the application of BDNF at different time windows before and during L-LTP in slices derived from BDNF knockout mice.

Since BDNF modulates long-term synaptic plasticity in hippocampus, one would expect that inhibition of BDNF signaling either by BDNF gene knockout, BDNF antisense oligonucleotides, or antibodies, should alter the performances of animals in behavioral tasks for learning and memory. BDNF (+/−) mice have been shown to exhibit deficits in spatial learning, as reflected by poor performance in the Morris water maze test (Linnarsson et al., 1997). Minichiello et al. (1999) have generated a line of conditional knockout mice with the deletion of TrkB gene restricted to the forebrain and hippocampus and occurs only during postnatal development. The adult −/− mice of this line show an impairment in LTP at hippocampal CA1 synapse as well as in complex learning behavior including Morris water maze and eight-arm radial maze, with no deficit in simple passive avoidance learning. Interestingly, +/− mice exhibit a partial reduction of LTP but appear behaviorally normal. These results suggest that CA1 LTP may need to be reduced below a

certain threshold before behavioral defects become apparent. In addition, the application of BDNF antibodies to the adult rat brain impairs learning and memory (Mu et al., 1999; Alonso et al., 2002a), whereas endogenous BDNF is required for spatial memory formation as well as retention in adult rats. For example, injection of the antisense BDNF oligonucleotide before and during memory consolidation markedly impairs memory retention performance, while injection 6 h post-training has no effect (Ma et al., 1998; Mizuno et al., 2000). The requirement of endogenous BDNF for both short-term and long-term memory (STM and LTM) formation has been demonstrated in rats on an inhibitory avoidance learning task, in which the infusion of anti-BDNF antibody impaired LTM when given 15 min before or 1 and 4 h after training, but not at 0 or 6 h post-training. These results suggest that two hippocampal BDNF-sensitive time windows are critical for LTM formation (Alonso et al., 2002b).

How does BDNF modulate L-LTP and long-term memory at the cellular and the molecular levels? Given that a key cellular change associated with long-term synaptic plasticity is synaptic growth, it is possible that synaptic morphological changes are induced, at least partially, by BDNF. Indeed, a number of experiments indicate that BDNF promotes axonal branching in the tectum and hippocampus (Cohen-Cory and Fraser, 1995; Danzer et al., 2002). Chronic exposure of the neurons in cortical slices to BDNF exerts profound effects on dendritic growth (McAllister et al., 1995, 1999; Lu, 2004). BDNF is also involved in activity-dependent refinement of synapses in the visual cortex (Cabelli et al., 1995, 1997). Of particular relevance is the morphological and geometrical change of dendritic spines in the hippocampus. The growth of dendritic spines and protrusions is enhanced in an NMDA-dependent manner at hippocampal CA1 synapses undergoing L-LTP (Engert and Bonhoeffer, 1999; Shi et al., 1999; Toni et al., 1999; Fukazawa et al., 2003). Treatment of hippocampal slices with BDNF increases the dendritic spine density in apical dendrites of CA1 pyramidal neurons (Tyler and Pozzo-Miller, 2001). Recent experiments from this laboratory indicate that BDNF regulation of dendritic spines is controlled by cAMP, a signaling molecule critically involved in L-LTP and long-term memory (Ji et al., unpublished observations). Thus, BDNF regulation of long-term synaptic plasticity may be mediated, at least in part, by its regulation of on dendritic spine formation.

An important yet unresolved issue is how BDNF secreted in the hippocampus during L-LTP and long-term memory. Is BDNF secreted from neurons or glial cells? While glia may secrete BDNF in a diffuse and constitutive manner, neurons are capable of secreting BDNF locally at tetanized synapses in an activity-dependent manner. Is pre-synaptic or post-synaptic compartment responsible for activity-dependent secretion of BDNF? Evidence from both sides has been obtained recently. Using optical imaging of a BDNF–GFP fusion protein in transfected hippocampal neurons in culture, BDNF has been shown to be packaged into secretory vesicles which are transported to somatodendritic compartment (Hartmann et al., 2001; Kojima et al., 2001). Upon high-frequency synaptic stimulation at the glutamatergic synapses, the BDNF–GFP particles rapidly disappear, suggesting secretion of BDNF–GFP from the synaptically localized secretory vesicles. On the other hand, Kohara et al. (2001) have demonstrated that activity-dependent secretion of BDNF occurs pre-synaptic terminals (Kohara et al., 2001). In the study, BDNF–GFP cDNA was injected in the nuclear of the living neurons, fluorescent spots representing BDNF was

shown to move not only from cell bodies to axons, but also from pre-synaptic terminals to post-synaptic neurons, in an activity-dependent manner. What is the source of Ca^{2+} that triggers BDNF secretion? A number of studies suggest that BDNF secretion is independent of extracellular Ca^{2+} but mediated by Ca^{2+} release from intracellular Ca^{2+} stores (Canossa et al., 1997; Griesbeck et al., 1999; Canossa et al., 2001; Gartner and Staiger, 2002). The post-synaptic secretion of BDNF has been shown to be critically dependent on Ca^{2+} influx into post-synaptic neurons via glutamate receptors or voltage-gated Ca^{2+} channels (Hartmann et al., 2001; Lever et al., 2001). Balkowiec and Katz (2002) have demonstrated that in hippocampal neurons, activity-dependent BDNF secretion requires Ca^{2+} influx through both N-type Ca^{2+} channels and Ca^{2+} mobilization from intracellular stores. Given that L-LTP could be divided into different stages (see above), it is tempting to speculate that BDNF is secreted at different loci through different mechanisms at temporally distinct stages. Shortly after tetanic stimulation, BDNF secretion could be triggered by Ca^{2+} influx at the post-synaptic dendrites or spines. Late in the process, BDNF could be secreted from the axonal terminals through a mechanism that involves Ca^{2+} release from intracellular stores. Further experiments are necessary to test this hypothesis.

What are the signaling mechanisms underlying BDNF regulation of L-LTP and long-term memory? Binding of BDNF to TrkB receptor rapidly activates its tyrosine kinase activity, which in turn triggers three major signaling pathways: MAPK, phosphatidylinositol 3-kinase (PI3-K) and phospholipase C-γ (PLC-γ) pathways (Segal et al., 1996; Huang and Reichardt, 2003). There is good evidence that activation of MAPK is critical for the full expression of BDNF-dependent forms of L-LTP, such as TBS- or forskolin-induced L-LTP, as well as long-term memory. Treatment of hippocampal slices with the MAPK kinase inhibitor U0126 or PD98059 before and during the application of L-LTP inducing tetanus markedly reduces the magnitude of L-LTP at Schaffer collateral-CA1 synapses, suggesting that MAPK activity is required for the induction of L-LTP (Patterson et al., 2001; Rosenblum et al., 2002). Application of PD98059 two min after the tetanus also attenuates L-LTP (Rosenblum et al., 2002). Patterson et al. have shown that forskolin, which they assumed induces the release of BDNF, rapidly activates MAPK, and this activation could not be blocked by TrkB–IgG. This result was interpreted as the lack of direct effect on BDNF on MAPK activation. However, application of BDNF has been shown to activate MAPK in the CA1 area of hippocampal slices (Gottschalk et al., 1999). Forskolin could activate MAPK through BDNF-independent mechanisms in the presence of TrkB–IgG. On the other hand, Patterson et al. (2001) provided a remarkably interesting phenomenon: TBS- and forskolin-induced nuclear translocation of phosphor-MAPK is dependent on TrkB ligand (BDNF). While the importance of MAPK translocation into the nucleus remains to be shown, it is very likely that the nuclear phosphorylation of CREB by the MAPK cascade contributes to BDNF regulation of L-LTP.

The correlation of BDNF and MAPK has also been demonstrated in some behavioral studies. For example, increased TrkB receptor phosphorylation appears to accompany the enhanced phosphorylation MAPK in dentate gyrus of rats undergoing Morris water maze training (Gooney et al., 2002). Infusion of anti-BDNF antibody into the hippocampal CA1 region impairs long-term memory as reflected in the inhibitory avoidance test, and this is accompanied with the decrease in MAPK activation. Conversely, intra-hippocampal administration of recombinant BDNF facilitates long-term memory, and this is also

accompanied with an enhancement in MAPK activation (Alonso et al., 2002b). However, behavioral studies could only provide indirect evidences for a role of MAPK phosphorylation in BDNF regulation of long-term memory.

Limited studies have been carried out on the role of PLC-γ in long-term hippocampal plasticity. Using knock-in techniques, Minichiello et al. (2002) have generated two lines of mice in which tyrosine residues in trkB that form a docking site for PLCγ (Tyr816) or Shc (Tyr515) upon phosphorylation have been selectively mutated. They showed that the L-LTP is severely impaired in the PLC-γ site mutants, but not in the Shc site mutants. They further provided the evidence that activation of PLC-γ is important for subsequent phosphorylation of Ca^{2+}-calmodulin IV (CaMKIV) and CREB (Minichiello et al., 2002). Thus, BDNF signaling through PLC-γ seems to be important for L-LTP at the Schaffer collateral-CA1 synapses. Furthermore, using the same Shc site mutant line, Korte et al. have found no difference in the induction-rate or magnitude of E-LTP and L-LTP induced by TBS or tetanic stimulation (Korte et al., 1998). They conclude that TrkB-coupled activation of MAPK is not required for hippocampal L-LTP. While these results suggest that Shc site of TrkB may not be required in L-LTP, one cannot rule out the importance of MAPK in BDNF regulation of the long-term synaptic plasticity. It is possible that multiple-train tetanic stimulation is sufficient to compensate the reduced MAPK activation due to impairment in the Shc site of TrkB.

Very few studies have examined the effect of PI3-K in L-LTP expression and long-term memory. Mizuno et al. recently carried out a behavioral study to address the role of TrkB-coupled PI3-K/Akt pathway in spatial learning and memory (Mizuno et al., 2003). Radial arm maze training in rats results in a significant increase in the BDNF mRNA expression in the hippocampus. Either infusion of the antisense BDNF oligodeoxynucleotide or chronic treatment with the PI3-K inhibitor wortmannin impairs spatial learning. More importantly, they have demonstrated that activation of the TrkB coupled PI3-K/Akt signaling pathway is associated with an enhancement in phosphorylated 4E-BP1 and a reduction in phosphorylated eEF-2, indicating an increase activity to translate mRNA into proteins. Taken together, these results suggest that activation of TrkB/PI3-K and protein synthesis signaling pathway by BDNF in the hippocampus is important for spatial memory.

Finally, how are the acute signaling events induced by BDNF translated into the long-term changes in synaptic structure and function that mediate L-LTP and long-term memory? One idea is that the signaling cascades lead to the expression of specific downstream target proteins. Several lines of experiments support this idea: (1) It is well established that BDNF rapidly induces the phosphorylation and activation of the transcription factor CREB (Finkbeiner et al., 1997). (2) There is also good evidence that BDNF, through activation of MAPK and PI3-K, enhances protein synthesis in neuronal dendrites (Aakalu et al., 2001; Takei et al., 2001). (3) The expression of a number of genes implicated in hippocampal plasticity is induced by BDNF. For example, BDNF, through activation of MAPK, enhances the expression of Arc, Zif268 and Homer 1a, proteins that have been strongly implicated in synaptic plasticity [Yin et al., 2002; Ying et al., 2002; Sato et al., 2001; Rosenblum et al., 2002; Alder et al., 2003]. Long-term treatment of hippocampal neurons also stimulates the expression of synaptic proteins synaptotagmin and rab3a (Tartaglia et al., 2001; Thakker-Varia et al., 2001). Biochemical experiments demonstrate that the long-term synaptic effects of BDNF in hippocampal slices are

dependent on cAMP and new protein synthesis (Tartaglia et al., 2001), characters reminiscent of L-LTP and long-term memory seen in many model systems. However, it remains to be established that BDNF regulates L-LTP through up-regulation of any of these genes. Since the expression of BDNF itself is up-regulated by L-LTP inducing stimulus, an alternative hypothesis is that BDNF itself is sufficient to elicit all the functional and structural changes of hippocampal synapses to sustain L-LTP. Consistent with this idea, treatment of hippocampal slices with BDNF elicits a dramatic increase in the number of dendritic spine of pyramidal neurons (Tyler and Pozzo-Miller, 2001), and increases the levels of synaptophysin and synaptobrevin (Tartaglia et al., 2001) and alters synaptic response to tetanic stimulation in a protein synthesis independent manner (Gottschalk et al., 1999). Most strikingly, BDNF applied after the delivery of tetanus is sufficient to maintain L-LTP in slices incubated in the protein synthesis inhibitor anisomycin during the entire course of recording (Pang et al., 2004). These results imply that BDNF is a key protein synthesis product, if not the only one, needed to carry on all the necessary functions for long-term modification of hippocampal synapses. Further work is necessary to assess this interesting supposition.

4. Regulation of L-LTP and memory by tPA

Is tPA one of the target genes responsible for L-LTP expression and long-term memory? An important criterion is whether tPA is expressed and/or secreted in an activity-dependent manner. Qian et al. have provided the first direct evidence for the enhancement of tPA mRNA expression by neuronal activity in hippocampus (Qian et al., 1993). With three different stimulation paradigms which independently evoke seizure, kindling or L-LTP, they found that the level of tPA mRNA is increased rapidly and markedly 1 h after the onset of stimulation in all cases. Moreover, both LTP and the enhancement in tPA expression are inhibited by the non-competitive NMDA receptor antagonist, MK801, suggesting that the induction of LTP and tPA transcription are correlated and require the activation of NMDA receptors (Qian et al., 1993). The idea that tPA is an immediate early gene is further supported by the demonstration that tPA is up-regulated in hippocampal CA1 and CA3 region after unilateral injection of kainic acid (Nagai et al., 1999; Salles and Strickland, 2002). Since tPA promoter contains a cAMP-responsive element (CRE) (Ohlsson et al., 1993), it is possible that transcription of the tPA gene is downstream from cAMP/PKA cascade and it is one of the target genes of CREB activation. Equally important, neuronal membrane depolarization has been shown to induce the secretion of tPA into extracellular space in the hippocampus in a Ca^{2+}-dependent manner (Gualandris et al., 1996; Nicole et al., 2001).

Direct evidence for the role of tPA in LTP expression and certain types of learning and memory comes from pharmacological studies using tPA and its inhibitors, and genetic studies using tPA transgenic and knockout mice. tPA mutant mice exhibit a selective impairment in L-LTP without defect in E-LTP in Schaffer collateral-CA1 and mossy fiber-CA3 synapses in the hippocampal slice (Frey et al., 1996; Huang et al., 1996; Calabresi et al., 2000). Disruption of tPA gene also prevents LTP in corticostriatum synapses (Centonze et al., 2002). Additionally, tPA mutant mice show impairments in context conditioning test

– a hippocampus-related behavioral task, and two-way active avoidance test – a striatum-dependent task (Calabresi et al., 2000), as well as in cerebellar motor learning (Seeds et al., 2003). Application of tPA inhibitors blocks L-LTP induced by tetanic stimulation, whereas treatment with tPA facilitates L-LTP induced by a single tetanus, which normally induces only E-LTP (Baranes et al., 1998). tPA activation is also required in cAMP/PKA agoinst-induced L-LTP in hippocampal slices, and such L-LTP is absent in slices treated with tPA inhibitors or those from tPA knock-out mice (Huang et al., 1996; Baranes et al., 1998). Furthermore, transgenic mice over-expressing tPA in postnatal neurons exhibit an enhancement and extension in LTP, as well as improvement in spatial learning and memory (Madani et al., 1999).

While a number of studies have implicated the role of tPA in L-LTP, the specific downstream target(s) of tPA involved in L-LTP remain to be established. So far, three targets have been suggested. One such target is NMDA receptor (Nicole et al., 2001). tPA selectively potentiates NMDA receptor-mediated calcium influx. Co-immunoprecipitation experiments indicate that NMDA receptor, particularly the NR1 subunit, forms a complex with tPA in cortical neurons. Application of NMDA results in the appearance of a shorter, presumably proteolytically cleaved fragment of NR1 on neuronal membrane, and this fragment is not seen in neurons treated with tPA inhibitor or from tPA−/− mice. These results were interpreted as tPA cleavage of the NR1. However, direct evidence for tPA cleavage of NMDA receptors is lacking. It is also difficult to imagine how cleavage of NR1 could lead to an enhancement of Ca^{2+} influx, and how this could lead to L-LTP.

Another potential target of tPA involved in L-LTP is its major substrate, plasminogen, which could be converted to the active protease plasmin through proteolytic cleavage by tPA (Plow et al., 1995). In the hippocampus, plasminogen mRNA and protein are expressed exclusively in neurons, primarily in pyramidal cell, and often seen in apical dendrites (Tsirka et al., 1997; Salles and Strickland, 2002). Plasminogen is secreted into extracellular space, possibly at synapses where it is converted to plasmin by tPA (Krystosek and Seeds, 1981; Salles and Strickland, 2002). Plasmin is one of the activators of metalloprotease precursors and could play a role in degrading certain extracellular matrix (He et al., 1989). It has been reported that plasmin enhances glutamate-evoked increase in Ca^{2+} influx in cultured hippocampal neurons through modulation of NMDA receptor (Inoue et al., 1994). However, mechanisms underlying plasmin regulation of synaptic plasticity are largely unexplored. Matsuki and his colleagues showed that plasmin facilitates LTP induction without affecting NMDA receptor in hippocampal slices (Mizutani et al., 1996). In another study, they showed that plasmin regulated LTP by degrading one of the components in the extracellular matrix – laminin (Nakagami et al., 2000). Pretreatment with 100 nM plasmin for 6 h was shown to impair the maintenance of LTP in organotypic cultures of hippocampal slices. Interestingly, this result appears to contradict the results of their previous study (Mizutani et al., 1996) as well as other studies which suggest that tPA is necessary for the maintenance of LTP expression (Frey et al., 1996; Huang et al., 1996; Baranes et al., 1998; Madani et al., 1999). The discrepancies may be due to different experimental paradigms such as the different durations of drugs perfusion; acute slices versus slice cultures, etc. We have recently obtained evidence which supports the hypothesis that tPA/plasmin plays an important role in L-LTP expression. Mice with the deletion of plasminogen gene express reduced L-LTP. In addition, plasmin appears to be

the downstream target of tPA since exogenous plasmin rescues L-LTP deficit in tPA knock-out mice (Pang et al., 2004). Altogether, these results suggest that tPA may contribute to L-LTP through the proteolytic cleaveage of plasminogen.

A provocative study suggests a third target of tPA: low-density lipoprotein receptor-related protein (LRP) (Zhuo et al., 2000). Rather than its proteolytic function, tPA may mediate L-LTP by binding to the endocytic receptor LRP. LRP is activated in L-LTP, but not E-LTP, since its inhibitor receptor-associated protein (RAP) only suppresses LTP induced by four-train tetanic stimulation, but not by one-train tetanus. In addition, tPA binding to LRP in hippocampal neurons enhances PKA activity, suggesting that cAMP/PKA pathway may be one of the intracellular events activated by tPA–LRP.

Taken together, one can imagine that L-LTP stimulus induces the expression of tPA, through the PKA/cAMP cascade and subsequent CREB activation. It also triggers the secretion of tPA, presumably at the extracellular synaptic cleft. While it is possible that tPA contributes to L-LTP by either proteolytic cleavage of NMDA receptor subunit or by binding to its potential receptor LRP, a more plausible mechanism is to convert its major substrate plasminogen to the active enzyme plasmin, which in turn activates downstream target proteins through proteolytic cleavage. It remains a great challenge to identify the specific target of the tPA/plasmin system that mediates the morphological and physiological change at the hippocampal synapses underlying L-LTP expression as well as long-term memory.

5. L-LTP and long-term memory in aging

Aging is tightly associated with memory impairments (for review see Rosenzweig and Barnes, 2003). Disruption of any one of the stages in the memory mechanism – acquisition, consolidation or retention – could lead to memory dysfunction. While deterioration in brain anatomy, physiology, plasticity and network dynamics could contribute to age-related memory deficits, the most reliable cellular system to address the effects of aging on long-term memory experimentally is hippocampal L-LTP. In fact, it has been shown that memory defects are correlated to the reduction in late-phase of L-LTP in aged animals (Bach et al., 1999; Hsu et al., 2002). Since synaptic plasticity is modulated by many factors, changes of these factors could result in the impairment in L-LTP and long-term memory. However, what discriminates L-LTP from E-LTP is its requirement of gene transcription and protein synthesis, leading to the modification of existing synapses and establishment of new synaptic contacts. Therefore, it is likely that L-LTP deficit in aged animals is due to impaired gene transcription and protein synthesis, rather than the factors involved in LTP induction such as Ca^{2+} influx through NMDA channels, activation of CaMKII, or AMPA receptor insertion, etc. Indeed, an age-related decrease in protein synthesis has been demonstrated in dentate gyrus (Kelly et al., 2000) as well as in other areas in the brain (Goldspink, 1988). In aged rats, impairment in memory and LTP is accompanied with the decrease in protein synthesis (Mullany and Lynch, 1997). Detailed analysis indicates that LTP-induce transcription of syntaxin 1B, subunits of NMDA receptor, and αCaMKII is significantly attenuated in aged animals (Davis et al., 2000b; Eckles-Smith et al., 2000; Clayton and Browning, 2001). Precisely which gene is responsible for the age-related defects in L-LTP remains unknown.

Impaired gene activation and transcription events could also be the consequence of defects in the intracellular signaling pathways. Compared with young mice, aged mice generally make more errors in spatial memory task and exhibit reduced L-LTP, but E-LTP in hippocampal CA1 region is intact. Pharmacological activation of adenylyl cyclase/cAMP pathway rescues L-LTP and facilitates long-term memory in aged mice, without enhancing normal L-LTP in young mice. Thus, age-related memory loss is associated with the disintegration of cAMP/PKA-dependent signaling pathway (Bach et al., 1999). An important finding is that age-related deficit in L-LTP and long-term memory could be restored by drugs that act to enhance the level of cAMP or to disrupt the function of pertussis toxin (PTX)-sensitive inhibitory G-protein (Bach et al., 1999; Hsu et al., 2002). Given the defects are reversible by drug application, it seems unlikely that the L-LTP and memory deficits in aged animals are caused by neuronal degeneration or gross structural abnormalities. In addition, Hsu et al. suggest that aging is associated with alterations of the balance of protein kinase/phosphatase activities, which in turn results in deficits in L-LTP as well as long-term memory (Hsu et al., 2002). Since cAMP/PKA/CREB phosphorylation is the key signaling pathway triggering gene transcription and protein synthesis required for L-LTP, it is possible that alternation of this signaling cascade plays a critical role in age-related L-LTP and memory deficits.

Given that both BDNF and tPA have been identified as immediate-early genes that are transcriptionally activated after L-LTP-inducing stimuli, and both are known to be critical for L-LTP, it is natural to speculate that they also take part in age-related impairments in plasticity and memory. Initial study using quantitative in situ hybridization and Northern blot analysis revealed no change in the expression or regional distribution of BDNF and trkB mRNAs in aged rats. These results argue against the role of BDNF/TrkB in age-related deficits in hippocampal synaptic plasticity or long-term memory (Lapchak et al., 1993). Later studies examined the expression of BDNF and trkB mRNAs as well as BDNF protein as a function of age and cognitive performance. BDNF mRNA in the pons, and trkB mRNA in many brain areas are significantly decreased during aging. There is also a marked reduction in the level of BDNF protein in septum, cerebral cortex, cerebellum, striatum and midbrain (Croll et al., 1998; Katoh-Semba et al., 1998). In addition, decreased trkB mRNA in the pons is correlated with impaired memory performance in aged rats, suggesting that the decrease in trkB mRNA with age is more widespread than decreases in BDNF (Croll et al., 1998). Immunohistochemistry study indicates that intensity of the BDNF-immunoreactivity declines significantly in cell bodies and dendrites of the neurons in the hippocampus of aged macaque monkeys (Hayashi et al., 2001). In studies using senescent rats, BDNF mRNA levels in the hippocampus correlate well with the performance in spatial learning (Schaaf et al., 2001). Moreover, in animals with impaired performance, the training-induced increase in BDNF mRNA is significantly attenuated. Little is known about the expression of tPA in aged brain. In one study, the seizure-induced increase in tPA mRNA is much less pronounced in aged hippocampus, as compared to young rats (Schmoll et al., 2001). The aged rat brains also respond more slowly to chemically induced seizure. Based on these limited and somewhat contradictory studies, however, it is difficult to conclude that age-related memory deficit is related to any alternations in the expression of BDNF, TrkB, or tPA.

6. Perspectives

Compared with E-LTP, much less is known about the cellular and molecular mechanisms underlying L-LTP. PKA and MAPK are widely accepted as the two key signaling pathways for L-LTP, but exactly how they translate the brief, high frequency stimulation into sustained structural and functional changes at the hippocampal synapses requires further investigation. Although substantial experimental data support the notion that protein synthesis and the transcription factor CREB are important for L-LTP, the specific CRE-driven gene products involved in different stages of L-LTP remain to be identified. An important area that deserves significant research efforts is the mechanisms by which synapse-specificity is achieved in L-LTP. Innovative genetic and cell biological approaches should be used to demonstrate in vivo that protein synthesis indeed occurs locally at the tetanized synapses. It will be both challenging and exciting to identify the nature of synaptic tag and plasticity factor(s). Finally, while both tPA and BDNF are known to be involved in L-LTP, their relationships and the underlying mechanisms are unclear. Given that tPA is an extracellular protease, it is possible that a proteolytic cleavage process may activate BDNF signaling system, leading to L-LTP expression. Indeed, recent study by this laboratory has demonstrated that tPA, by activating extracellular protease plasmin, converts the precursor proBDNF to mature BDNF (mBDNF), and such conversion is critical for L-LTP (Pang et al., 2004). mBDNF, but not cleavage-resistant proBDNF, converts E-LTP to L-LTP and rescues the L-LTP deficits in the presence of the protein synthesis inhibitor anisomycin. Moreover, mBDNF rescues L-LTP in tPA and plasminogen knockout mice. Biochemical experiments confirm that plasmin, but not tPA, cleaves proBDNF to form mBDNF. Genetic and pharmacological experiments indicate that mBDNF is downstream of plasmin, which is downstream of tPA, in L-LTP expression. Taken together, these results reveal a physiological role of proBDNF–mBDNF conversion by extracellular proteases in the brain, and provide an unexpected, mechanistic link between tPA and BDNF in L-LTP expression. Further experiments are necessary to determine the specific stage(s) by which tPA and BDNF regulates L-LTP, the molecular targets of tPA/mBDNF that mediate the long-term structural and functional changes at the hippocampal synapses, and how BDNF/tPA achieves synapse-specific modulation.

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