Research

Age-related enhancement of a protein synthesis-dependent late phase of LTP induced by low frequency paired-pulse stimulation in hippocampus

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Protein synthesis-dependent late phase of LTP (L-LTP) is typically induced by repeated high-frequency stimulation (HFS). This form of L-LTP is reduced in the aged animal and is positively correlated with age-related memory loss. Here we report a novel form of protein synthesis-dependent late phase of LTP in the CA1 region of hippocampus induced by a brief 1-Hz paired-pulse stimulation (PP-1 Hz, 1 min). In contrast to L-LTP induced by HFS, the late phase of PP-1 Hz LTP does not exist in young adult animals. Rather, it emerges and becomes enhanced in an age-related way. Thus, in 1.5- to 2-mo-old mice, a brief PP-1 Hz stimulation induces only a short lasting LTP, decaying to baseline in about 90 min. By contrast, PP-1 Hz stimulation induces an enduring and protein synthesis dependent LTP in 12- to 18-mo-old mice. The PP-1 Hz-induced L-LTP is dependent on NMDA receptor activation, requires voltage-dependent calcium channels, and is modulated by dopamine D1/D5 receptors. Because memory ability declines with aging, the age-related enhancement of L-LTP induced by PP-1 Hz stimulation indicates that this form of L-LTP appears to be inversely correlated with memory ability.

Long-term synaptic plasticity in the hippocampus is thought to be a cellular substrate for learning and memory. Two families of long-lasting changes in the excitatory synaptic connections in the CA1 region of hippocampus have been identified: long-term synaptic potentiation (LTP) and long-term synaptic depression (LTD). Although LTP is commonly induced by high-frequency stimulation (HFS) and LTD is induced by low-frequency stimulation, LTP can also be induced by low-frequency stimulation. For instance, whereas a prolonged 5-Hz stimulation induces LTD, a brief 5-Hz stimulation elicits LTP (Thomas et al. 1996; Zakharenko, et al. 2002; Morozov et al. 2003; Huang and Kandel 2005).

Stimulation at a frequency of 1 Hz typically induces LTD in the Schaffer Collateral pathway of young animals. However, 1 Hz of paired pulses (PP) (1 Hz) stimulation (50-msec PP interval, 900 pairs, 1 Hz) can elicit even stronger LTD in the hippocampus of adult animals (Kemp and Bashir 1999; Huber et al. 2000, 2002; Kemp et al. 2000; Lee et al. 2003; Alarcon et al. 2004). Because the strength of synaptic transmission can be bidirectionally regulated by different patterns of presynaptic stimulation (Bear et al. 1987; Artola and Singer 1993; Shouval et al. 2002; Castellani et al. 2005), we asked, "Can PP-1 Hz stimulation also induce LTP?" We examined this question in the CA1 region of hippocampus by using different durations of PP-1 Hz stimulation (1 min–15 min). We find that the synaptic plasticity induced by PP-1 Hz stimulation is bidirectional, depending on the total number of stimulation pulses. Although prolonged PP-1 Hz stimulation (15 min) induced LTD, a brief PP-1 Hz stimulation (1 min) induces synaptic potentiation in adult animals. Moreover, LTP induced by PP-1 Hz stimulation is age dependent. PP-1 Hz stimulation induced an

early phase of LTP (E-LTP) only in young adult mice (1.5–3 mo) in which repeated HFS induces a substantial late phase of LTP (L-LTP). An enduring and protein synthesis-dependent late phase induced by PP-1 Hz stimulation only emerges from 6–7 mo. In 18-mo-old mice, the magnitude of L-LTP induced by PP-1 Hz stimulation is significantly greater than that in 3-mo-old mice.

Aging is associated with memory decline and the magnitude of conventional L-LTP is reduced in aged animals (Bach et al. 1999). The age-related increase of L-LTP induced by a brief PP-1 Hz stimulation, we have found here, is opposite to the age-related decrease of L-LTP induced by HFS (four trains of 100 Hz). This finding indicates that L-LTP induced by PP-1 Hz stimulation appears to be a form of LTP that is negatively coupled with age-related memory loss and may, therefore, have a unique, perhaps negative, role in memory storage.

Results

A brief PP-1 Hz stimulation induces LTP

In studies of LTD, a number of investigators have encountered the interesting phenomenon that excitatory postsynaptic potentials (EPSPs) first exhibit a transient potentiation during 1 Hz or PP-1 Hz stimulation, followed by a long-lasting depression. This synaptic potentiation lasts about 1 min (Mulkey et al. 1993, 1994; Kemp and Bashir 1999; Kemp et al. 2000; Alarcon et al. 2004; Milner et al. 2004). We asked, "Can this transient potentiation be maintained by reducing the total pulse number of PP-1 Hz stimulation?" We first examined synaptic plasticity induced by PP-1 Hz stimulation in young adult mice (6–8 wk) and we found that the changes of synaptic plasticity are bidirectional, depending on the duration (the pulse number) of this stimulation. Fifteen minutes of 1-Hz PP (900 pairs) stimulation induced LTD ($70 \pm 2\%$, $n = 6$) (Fig. 1A) and 10 min of PP-1 Hz stimulation (600 pairs) induced a weaker depression ($81 \pm 6\%$, $n = 6$) (Fig. 1B). When the duration of PP-1 Hz stimulation was reduced to 3

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Figure 1. A brief PP-1 Hz stimulation induces LTP (in 6- to 8-wk-old mice). (A) Fifteen minutes of PP-1 Hz stimulation (900 pairs) induced LTD. (B) Ten minutes of PP-1 Hz stimulation (600 pairs) induced a weak synaptic depression. (C) Three minutes of PP-1 Hz stimulation (180 pairs) induced a weak synaptic potentiation. (D) A brief PP-1 Hz stimulation (1 min, 60 pairs) induced LTP. (E) A summary of synaptic strength 15 min after PP-1 Hz stimulation (1 min–15 min). (F) Representative EPSPs waveforms before, during, and after PP-1 Hz stimulation (the smaller EPSP is before PP-1 Hz). Calibration: 2 mV, 10 ms.

min (180 pairs), a moderate synaptic potentiation was elicited ($115 \pm 8\%$, $n = 8$) (Fig. 1C). Finally, when a briefer PP-1 Hz stimulation (1 min, 60 pairs) was applied, a substantial synaptic potentiation was induced ($149 \pm 6\%$, $n = 6$) (Fig. 1D). A summary of synaptic strength (15 min after PP-1 Hz stimulation) as a function of the duration of PP-1 Hz stimulation is illustrated on Figure 1E. During the period of PP-1 Hz stimulation (after 20–30 pairs), complex spike bursts superimposed on field EPSPs (fEPSPs) (Thomas et al. 1998; Winder et al. 1999) were regularly detected (Fig. 1F).

Age-related emergence and enhancement of L-LTP induced by PP-1 Hz stimulation

Most previous studies of LTD induced by PP-1 Hz stimulation were performed in young animals ranging from 6 d to 100 d postnatal. In these animals, studies focused on the early component of synaptic change (15–50 min after 1 Hz stimulation) (Kemp and Bashir 1999; Kemp et al. 2000; Huber et al. 2000, 2002). However, these studies have not examined whether PP-1 Hz stimulation gives rise to an age-related change in protein synthesis-dependent long-lasting synaptic plasticity. We therefore asked, “Can PP-1 Hz stimulation induce an L-LTP? What is the difference in L-LTP between mice of different ages?” We found that PP-1 Hz stimulation (1 min) induced only an E-LTP in 6- to 8-wk-old mice; it relaxed to baseline in about 90 min ($103 \pm 4\%$, 2.5 h after PP-1 Hz stimulation, $n = 9$) (Fig. 2A). When the age of mice was increased to 3 mo, PP-1 Hz stimulation also induced L-LTP but at a very low level ($124 \pm 11\%$ 2.5 h after PP-1 Hz stimulation, $n = 7$) (Fig. 2B). However, in 6- to 7-mo-old mice, a substantial late phase of LTP emerged. Two and a half hours after PP-1 Hz stimulation, LTP was $164 \pm 7\%$ ($n = 8$), which is significantly different from the LTP in 1.5- to 2-mo-old mice ($P < 0.01$) (Fig. 2C). In 12- and 18-mo-old mice, PP-1 Hz induced even greater L-LTP ($178 \pm 9\%$, $n = 9$ and $181 \pm 12\%$, $n = 7$) (Fig. 2D,E), which was significantly different from the L-LTP in 3-mo-old mice ($P < 0.01$), but is not significantly larger than L-LTP in 6- to 7-mo-old mice ($P > 0.05$). A histogram of L-LTP (2.5 h after PP-1 Hz stimulation) in different ages is shown in Figure 2F. There is no significant difference of E-LTP (30 min after PP-1 Hz) between 6- to 8-wk ($150 \pm 9\%$), 3-mo ($151 \pm 12\%$), 6-mo ($161 \pm 8\%$), 12-mo ($159 \pm 10\%$), and 18-mo-old ($169 \pm 11\%$, Fig. 2G) mice. There is also no significant difference of input–output curves between 3-mo-old and 12- to 18-mo-old mice (Fig. 2H).

L-LTP induced by HFS is dependent on new protein synthesis. Is the L-LTP induced by PP-1 Hz stimulation also de-

pendent on new protein synthesis? Since L-LTP induced by PP-1 Hz is fully developed in 6- to 7-mo-old mice, we examined the requirement for protein synthesis in 6- to 7-mo-old mice. Here, we first demonstrate that the L-LTP induced by PP-1 Hz stimulation is long lasting. As shown in Figure 3A, LTP 3 h ($156 \pm 11\%$) and 5 h ($159 \pm 6\%$) after PP-1 Hz stimulation is not significantly different from LTP at 1 h ($150 \pm 7\%$, $n = 6$, $P > 0.5$). The protein synthesis inhibitor emetine (100 μ M) was added to the bath 30–60 min before PP-1 Hz stimulation. In the presence of emetine, LTP was $112 \pm 8\%$ ($n = 7$) two and a half hours after PP-1 Hz

Figure 2. Age-related enhancement of late-phase LTP induced by PP-1 Hz stimulation. (A) Brief PP-1 Hz stimulation (1 min) induced E-LTP only in 6- to 8-wk-old mice. (B) PP-1 Hz stimulation (1 min) induced a weak L-LTP in 3-mo-old mice. (C) PP-1 Hz stimulation (1 min) induced a substantial L-LTP in 6- to 7-mo-old mice. (D) PP-1 Hz stimulation (1 min) induced L-LTP in 12-mo-old mice. (E) PP-1 Hz stimulation (1 min) induced L-LTP in 18-mo-old mice. (F) A histogram showing the magnitude of L-LTP (2.5 h after PP-1 Hz stimulation) in mice of different ages. (G) A histogram showing the magnitude of E-LTP (30 min after PP-1 Hz stimulation) in mice of different ages. (H) Input-output curves in 3-mo-old and 12- to 18-mo-old mice. *Insets* in A, B, C, D, and E show sample traces of fEPSPs 10 min before and 2.5 h after PP-1 Hz stimulation. Calibration: 3 mV, 5 ms.

stimulation, which was significantly different from L-LTP in control slices ($161 \pm 6\%$, $n = 8$, $P < 0.01$) (Fig. 3B). Rapamycin, an inhibitor of mammalian target of rapamycin (mTOR) that is criti-

cally involved in local protein synthesis in dendrites of neuron, also blocks L-LTP induced by HFS (Tang et al. 2002). We therefore next examined the effect of rapamycin (0.5 μ M) on PP-1 Hz L-

LTP and found that it significantly reduced the L-LTP induced by PP-1 Hz stimulation (Rapamycin: $125 \pm 5\%$, $n = 6$; Control: $162 \pm 6\%$, $n = 6$, 2.5 h after PP-1 Hz stimulation, $P < 0.01$) (Fig. 3C). Different from emetine, rapamycin has only a modest inhibitory effect on total protein synthesis (24%) in hippocampal slices (Cracco et al. 2005). The weaker depression of PP-1 Hz L-LTP in slices treated with rapamycin indicates that local protein synthesis may play a modest role in this form of L-LTP.

PP-1 Hz L-LTP is dependent on *N*-methyl-D-aspartate (NMDA) receptor and voltage-dependent calcium channel (VDCC)

L-LTP induced by repeated HFS depends on the activation of NMDA receptors (Huang et al. 2005). To determine whether L-LTP induced by a brief PP-1 Hz stimulation (1 min) requires the activation of NMDA receptor, we examined the effect of NMDA antagonist D-AP5 (50 μM) in 6- to 7-mo-old mice. We found that it completely blocked both early and late phase of PP-1 Hz LTP (Control: $160 \pm 7\%$, $n = 6$; APV: $107 \pm 8\%$, $n = 7$, $P < 0.01$) (Fig. 4A). The results indicate that the L-LTP induced by PP-1 Hz stimulation, like several other forms of L-LTP, requires the activation of NMDA receptors. A prolonged PP-1 Hz stimulation (15 min) induces LTD that requires the activation of metabotropic glutamate receptors (mGluRs) (Kemp and Bashir 1999; Huber et al. 2000; Klempisch et al. 2001). Is L-LTP induced by a brief PP-1 Hz stimulation (1 min) also dependent on mGluR? To address this question, we examined the effect of mGluR antagonist LY341495 (inhibit both mGluR I and II) (Kingston et al. 1998; Kemp and Bashir 1999; Wu et al. 2004). In contrast to D-APV, LY341495 (50 μM) failed to block L-LTP (LY341495: $178 \pm 3\%$, $n = 6$; Control: $168 \pm 5\%$, $n = 6$, 2.5 h after PP-1 Hz stimulation, $P > 0.5$) (Fig. 4B), indicating that LTP induced by PP-1 Hz stimulation is independent of the activation of mGluR (Type I and II) receptors in our experimental condition. Previous studies have demonstrated that the synaptic plasticity induced by PP-1 Hz or single 1-Hz stimulation could be either NMDA-dependent or mGluR-dependent, depending on age of the animal, the experimental conditions (temperature, components of perfusion solution), and the stimulation parameters (Oliet et al. 1997; Huber et al. 2000, 2002; Kemp et al. 2000; Lee et al. 2003; Malenka and Bear 2004). Under a different experimental condition (37°C), LTP induced by a single 1-Hz stimulation in rat slices treated with GABA inhibitor could become mGluR dependent (Lanté et al. 2005).

VDCCs are also importantly involved in the maintenance of late-phase LTP (Impey et al. 1996; Huang et al. 2004). We therefore asked, "Is PP-1 Hz LTP dependent on VDCCs?" We applied PP-1 Hz (1 min) in the presence of VDCC inhibitor nifedipine (15 μM) in slices from 6- to 7-mo-old mice. This treatment did not affect the early component of PP-1 Hz LTP but significantly reduced the LTP 2.5 h after the PP-1 Hz stimulation (nifedipine: $112 \pm 10\%$, $n = 6$; Control: $166 \pm 7\%$, $n = 6$; $P < 0.01$) (Fig.

Figure 3. Protein synthesis inhibitors block L-LTP induced by PP-1 Hz stimulation. (A) L-LTP induced by PP-1 Hz stimulation lasts up to 5 h. *Insets* are sample traces at 1 h, 3 h, and 5 h after PP-1 Hz stimulation. Calibration: 3 mV, 5 ms. (B) Emetine (100 μM) blocked L-LTP induced by PP-1 Hz stimulation. (○) Control; (●) Emetine. The addition of emetine did not alter the baseline EPSPs (Δ , $n = 5$). (C) Rapamycin (0.5 μM) depressed L-LTP induced by PP-1 Hz stimulation. (○) Control; (●) Rapamycin. The addition of rapamycin did not alter the baseline EPSPs (Δ , $n = 5$). *Insets* in B and C show sample traces of EPSPs 10 min before and 2.5 h after PP-1 Hz stimulation. Calibration: 2 mV, 5 ms.

Figure 4. LTP induced by PP-1 Hz stimulation is dependent on NMDA receptor activation. (A) NMDA receptor antagonist D-APV (50 μ M) completely blocked the LTP induced by PP-1 Hz stimulation. (○) Control; (●) D-APV. (B) mGluR antagonist LY341495 did not alter the expression of LTP induced by PP-1 Hz stimulation. (○) Control; (●) LY341495. (C) VDCC antagonist Nifedipine selectively blocked the late phase of LTP induced by PP-1 Hz stimulation. (○) Control; (●) Nifedipine. (D) Nifedipine did not block the E-LTP induced by PP-1 Hz stimulation in 6- to 8-wk-old mice. Insets in B, C, and D, show sample traces of EPSPs 10 min before and 2.5 h after (A, 90 min after) PP-1 Hz stimulation. Calibration: 2mV, 5ms.

4C). By contrast, nifedipine did not block the E-LTP induced by PP-1 Hz stimulation in 6- to 8-wk-old mice (Fig. 4D).

The population complex spikes superimposed on the fEPSPs (Fig. 1F) during low-frequency stimulation was reported to be involved in the induction and early maintenance of LTP (Thomas et al. 1998; Winder et al. 1999). Is there any age-related difference of complex spikes evoked by PP-1 Hz stimulation? We counted the number of population complex spikes during PP-1 Hz stimulation (1 min) and no significant difference in the total number of complex spikes was found between young and aged mice (12–18 mo: $177 \pm 12\%$, $n = 8$; 6–18 wk: $152 \pm 25\%$, $n = 8$, $P > 0.05$). This result indicates that the difference of LTP induced by low-frequency stimulation in young and aged mice is unrelated to the number of complex spikes (Geoffrey et al. 2002).

PP-1 Hz L-LTP is modulated by dopamine receptors and protein kinase A (PKA)

Dopamine receptors are importantly involved in the maintenance of L-LTP induced by HFS (Frey et al. 1991; Huang and Kandel 1995; Otmakhova and Lisman 1996; Matthies et al. 1997). Antagonists of D1/D5 dopamine receptors depress the L-LTP induced by repeated 100-Hz stimulation in the CA1 region of the hippocampus (Frey et al. 1991; Huang and Kandel 1995). What is

the role of D1/D5 receptor in the L-LTP induced by PP-1 Hz stimulation? We first examined the effect of dopamine antagonist in 6- to 7-mo-old mice. In the presence of SCH23390 (2 μ M), LTP was $114 \pm 5\%$ ($n = 7$) two and a half hours after PP-1 Hz stimulation, which is significantly smaller than the LTP in the control experiment ($169 \pm 9\%$, $n = 7$, $P < 0.01$) (Fig. 5A). However, SCH23390 did not block E-LTP induced by PP-1 Hz stimulation in 6- to 8-wk-old mice (Fig. 5B). We next examined the effect of D1 agonist. E-LTP induced by a single 100-Hz stimulation could be switched to L-LTP by coapplication of D1 agonist (Bach et al. 1999; Huang et al. 2005). Can E-LTP induced by PP-1 Hz stimulation in young adult animals also be switched to L-LTP by adding D1 agonist? As shown in Figure 5C, even in the presence of D1 agonist 6-Bromo-APB (10 μ M), PP-1 Hz stimulation still induced only E-LTP in 6- to 8-wk-old mice (APB: $108 \pm 9\%$, 90 min after PP-1 Hz stimulation, $n = 7$; Control: $101 \pm 7\%$, $n = 6$, $P > 0.5$) (Fig. 5C). Similarly, the application of dopamine (200 μ M), which activates both D1 and D2 receptor, did not facilitate the LTP in 6- to 8-wk-old mice (Dopamine: $104 \pm 8\%$, $n = 6$; Control: $104 \pm 7\%$, $n = 6$, $P > 0.5$; data not shown). These experiments indicate that although both PP-1 Hz LTP and HFS LTP require the mediation of dopamine receptors, the recruitments of dopamine receptors by these two protocols are different.

Figure 5. L-LTP induced by PP-1 Hz stimulation is mediated by D1/D5 receptors. (A) D1/D5 receptor antagonist SCH23390 selectively blocked the L-LTP induced by PP-1 Hz stimulation in 6- to 7-mo-old mice. (○) Control; (●) SCH. (B) SCH23390 did not block the E-LTP induced by PP-1 Hz stimulation in 6- to 8-wk-old mice. (C) D1/D5 agonist 6-bromo-APB did not facilitate the L-LTP induced by PP-1 Hz stimulation in 6- to 8-wk-old mice. (D) PKA inhibitor KT5720 blocked the L-LTP induced by PP-1 Hz stimulation in 6- to 7-mo-old mice. (○) Control; (●) KT5720. Insets in A, B, C, and D are sample traces of EPSPs 10 min before and 2.5 h after PP-1 Hz stimulation. Calibration: 2mV, 5 ms.

D1/D5 receptor's activation is coupled with cAMP/PKA via Gs protein. The protein synthesis-dependent L-LTP induced by HFS is mediated by PKA signaling pathway (Abel et al. 1997; Huang et al. 2005). Is the L-LTP induced by PP-1 Hz stimulation dependent on PKA? We examined the effect of PKA inhibitor KT5720 (1 μ M) and found that the late phase, but not the early phase, of PP-1 Hz LTP was significantly depressed (KT5720: $103 \pm 2\%$, 2.5 h after PP-1 Hz stimulation, $n = 6$; Control: $162 \pm 7\%$, $n = 6$, $P < 0.01$) (Fig. 5D). These results suggest that PKA-mediated protein synthesis may also underlie the maintenance of the late component of PP-1 Hz LTP.

The results above indicate that although the late phase of PP-1 Hz LTP and HFS LTP are different in aged animals, they have common features. This raised the question: Can these two forms of L-LTP occlude each other? To address this issue, we performed an "occlusion" experiment in 6- to 7-mo-old mice. Four trains of 100 Hz (1 sec) were applied first to induce a conventional L-LTP. Forty-five minutes after the induction of LTP, the stimulus intensity was reduced to reach baseline level and PP-1 Hz stimulation (1 min) was applied. As shown in Figure 6, PP-1 Hz stimulation still induced L-LTP after HFS L-LTP, which was not significantly different from that in the control experiment without priming 4 trains of tetanus (Control: $162 \pm 12\%$, $n = 6$; after HFS: $143 \pm 9\%$, $n = 5$, 2.5 h after PP-1 Hz stimulation, $P > 0.05$). These experiments indicate that L-LTP induced by PP-1 Hz stimulation shares some, but not all, of the component of L-LTP induced by HFS stimulation.

Discussion

An age-related enhancement of L-LTP induced by PP-1 Hz stimulation

L-LTP is different from E-LTP in that it is enduring and dependent on new protein synthesis. Whereas L-LTP is typically induced by repeated and spaced HFS (3 or 4 trains of 100 Hz) (Huang and Kandel 1994; Impey et al. 1996; Abel et al. 1997; Nayak et al. 1998; Kelleher III et al. 2004), L-LTP can also be induced by theta bursts (bursts at 100 Hz, repeated in 5 Hz) (Nguyen and Kandel 1997; Woo et al. 2000; Dudek and Fields 2002; Alarcon et al. 2004), heterosynaptic pairing (Huang et al. 2004), and theta frequency stimulation (5 Hz, 30 sec) (Morozov et al. 2003; Huang et al. 2005). We here report a new form of L-LTP that is induced by a brief 1-Hz of paired pulse (1 min) stimulation. In contrast to all other forms of L-LTP, which exist in young adult animals, L-LTP induced by PP-1 Hz stimulation does not exist in young adult animals.

LTP induced by HFS emerges 7–14 d postnatally. In 6- to 12-wk-old mice, a protein synthesis-dependent IL-LTP induced by repeated HFS is well established (Abel et al. 1997; Barco et al. 2002; Pittenger et al. 2002; Alarcon et al. 2004; Kelleher III et al. 2004). The expression of LTP induced by HFS exhibits an age-related reduction (for review, see Rosenzweig and Barnes 2003; Lynch 2004). The magnitude of L-LTP induced by HFS in 18-mo-old mice is significantly smaller than that in 3-mo-old mice (Bach et al. 1999). In contrast to the L-LTP induced by HFS, we here

Figure 6. HFS L-LTP does not occlude L-LTP induced by PP-1 Hz stimulation. (○) L-LTP induced by PP-1 Hz stimulation without priming of HFS L-LTP. (●) PP-1 Hz L-LTP after L-LTP induced by four trains of 100 Hz tetanus (1 sec). Insets show sample traces of EPSP before and 2.5 h after PP-1 Hz stimulation in control and occlusion experiments. Calibration: 3 mV, 10 ms.

demonstrate that the magnitude of L-LTP induced by PP-1 Hz stimulation is significantly larger in 18-mo-old mice than that in 3-mo-old mice. Why is L-LTP induced by PP-1 Hz stimulation coupled with aging in an opposite direction? Recent findings suggest that with increasing age, some signaling pathways are down-regulated, while others are up-regulated. For instance, Ca^{2+} current influx through L-type channel (VDCC) increases in aged animals (Campbell et al. 1996; Thibault and Landfield 1996). An increase of the early phase of VDCC LTP (but decrease of NMDA LTP) in aged animals induced by 200 Hz tetanus has been reported (Shankar et al. 1998). Since VDCC antagonist selectively blocks the late component of PP-1 Hz LTP, the age-related alteration of Ca^{2+} influx through L-type channels may contribute to the age-related enhancement L-LTP induced by PP-1 Hz stimulation. The amplitude and duration of postsynaptic Ca^{2+} elevation is essential for up- and down-regulation of synaptic weight (Mizuno et al. 2001; Shouval et al. 2002). HFS and PP-1 Hz stimulation may elicit different dynamic changes of postsynaptic Ca^{2+} in an aged animal, resulting in distinct changes of L-LTP.

Autoradiographic studies have revealed that there are age-related changes in the density of D1/D5 receptors and dopamine levels in hippocampus (Luine et al. 1990; Amenta et al. 2001). Since D1/D5 antagonists selectively block the late component of PP-1 Hz LTP, which emerges in an age-related way, this form of L-LTP appears to be related to the age-related changes of D1/D5 receptor and dopamine level. The blockade of PP-1 Hz L-LTP by PKA inhibitor suggests that dopamine receptors might also be involved and mediated through cAMP/PKA signaling pathway. Although dopamine antagonists block both HFS L-LTP and PP-1 Hz L-LTP, the effects of dopamine agonists on these two forms of L-LTP are distinctly different. D1 receptor agonists facilitate L-LTP induced by 100-Hz stimulation (Bach et al. 1999; Otmakhva and Lisman 1996; Huang et al. 2005), but D1 agonists do not facilitate L-LTP induced by PP-1 Hz stimulation in a young animal. Thus, although both HFS L-LTP and PP-1 Hz L-LTP are influenced by activation of dopamine receptors, the dopaminergic effects in these two forms of L-LTP are different.

The long-term maintenance of both HFS LTP and PP-1 Hz LTP requires new protein synthesis. However, the different dynamic changes of postsynaptic Ca^{2+} induced by HFS and PP-1 Hz stimulation may recruit different downstream signaling cascades or the same signaling cascade in opposite directions. In this way, PP-1 Hz stimulation may recruit distinct sets of proteins and transcription factors that are different from those recruited by HFS.

These proteins may be negatively coupled with the synaptic strength. Indeed, an increase (rather than decrease) in expression of majority genes has been reported to be associated with the impairment of memory in the middle age (15 mo) mice, indicating negative regulation at the transcriptional level on the aging-related memory deficit in hippocampal neurons (Verbitsky et al. 2004). The age-related enhancement of protein synthesis-dependent L-LTP in this study could be related to such negative regulation. As a corollary, our studies indicate that it is essential to appreciate that LTP is not a single phenomenon but a large family of synaptic plasticity produced by different patterns of stimulation and that as result even slight changes in the induction protocol may have profound effects in the signaling pathway that are recorded.

Different forms of LTP may correlate with memory in distinct ways

A considerable body of evidence supports the idea that LTP in the Shaffer Collateral pathway is involved in the storage of spatial memory (for review, see Martin and Morris 2002; Lynch 2004). It is generally believed that the induction or expression of LTP in this pathway is positively correlated to the ability to store spatial memory. A reduction of LTP is frequently correlated with deficits in learning and memory (Abel et al. 1997; Kang et al. 2001; Pittenger et al. 2002; Kelleher III et al. 2004; Wood et al. 2005) and an enhancement of LTP is often associated with facilitation of learning and memory (Manabe et al. 1998; Madani et al. 1999; Tang et al. 1999; Nakamura et al. 2001). In previous studies of age-related change in LTP, the maintenance of LTP is markedly impaired with age (for review, see Rosenzweig and Barnes 2003; Lynch 2004) and memory deficits in an aged animal are associated with the reduction of LTP (Bach et al. 1999; Geoffrey et al. 2002; Hsu et al. 2002). We find here however that the late phase of PP-1 Hz LTP increases in aged mice. This form of L-LTP is quite different from the L-LTP induced by HFS. Since hippocampus-based learning and memory decreases from 3-mo-old mice to 15- to 18-mo-old mice (Bach et al. 1999; Verbitsky et al. 2004; Praag et al. 2005), the increase of PP-1 Hz L-LTP from 3-mo- to 18-mo-old mice, we find here, indicates that this form of L-LTP appears to be inversely coupled to the memory deficits and blockade of this form of L-LTP may facilitate memory. Earlier findings showed that inhibitors of L-type calcium channels can ameliorate learning deficits in aged animals (Deyo et al. 1989; Woodruff-Pak et al. 1997; Veng et al. 2003). These effects may be correlated with the blockade of increased PP-1 Hz L-LTP in aged animals in the presence of L-type calcium channels inhibitors.

Our finding demonstrates that different forms of L-LTP induced by different protocols must have distinct functional significance to learning and memory. Animals with poor memory may have a poor L-LTP induced by HFS but have a better L-LTP induced by PP-1 Hz stimulation. Delineation of the distinct coupling of memory with these two different forms of LTP and their underlying mechanism may provide a deeper insight into memory related synaptic plasticity in the hippocampus.

Materials and Methods

Transverse slices (400 μm) of acutely dissected mouse hippocampus were prepared from 6- to 8-wk-, 3-mo-, 6- to 7-mo-, 12-mo-, and 18-mo-old mice (C57/B6). Slices were maintained in an interface chamber with continuous perfusion of artificial cerebrospinal fluid (aCSF) at 2 mL/min and bubbled with 95% O_2 and 5% CO_2 . The composition of aCSF was as follows (in mM): 124 NaCl, 1.3 MgSO_4 , 4 KCl, 1.0 NaH_2PO_4 , 2 CaCl_2 , 26 NaHCO_3 , and 10 D-glucose. The temperature of the slice was maintained at 28°C. Experiments were started 2–3 h after the slice dissection.

Extracellular recordings were made using aCSF-filled glass

electrodes (1–3 M). fEPSPs were recorded in the stratum radiatum of the CA1 region. Stimuli were delivered through bipolar stainless steel electrodes (25 μ M wire diameter, FHC). The baseline responses were elicited one per minute (0.017 Hz). Stimulation intensity was adjusted to evoke fEPSP amplitudes that were about 30%–50% of maximal size. The intensity and pulse duration of stimulation for baseline sampling and LTP/LTD induction were the same (0.05 ms). LTP/LTD were elicited by paired stimuli (30-ms inter-stimulus interval) at 1 Hz (60 pairs to 900 pairs). Any changes in synaptic strength were expressed relative to normalized baseline (mean $\pm$ SEM). Statistical comparisons were performed using Student's *t*-test.

When used, pharmacological agents were applied via the bath medium. Drugs used were: KT5720 (Biomol) dissolved in dimethyl sulfoxide (DMSO), Emetine (Sigma) in DMSO, D-APV (D(-)-2-amino-5-phosphonopentanoic acid) (Sigma), Rapamycin (Calbiochem) in DMSO, Nifedipine (Sigma) dissolved in DMSO, SCH23390 (Sigma), 6-bromo-APB (Sigma) in DMSO, LY341495 (Tocris) dissolved in equimolar NaOH. Drugs were made and stored as concentrated stock solution and diluted 1000-fold when applied to the perfusion solution. The final concentration of DMSO is 0.1%. In all control experiments for drugs dissolved in DMSO, 0.1% DMSO was used.

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