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Developmental regulation of the late phase of long-term potentiation (L-LTP) and metaplasticity in hippocampal area CA1 of the rat

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Cao G, Harris KM. Developmental regulation of the late phase of long-term potentiation (L-LTP) and metaplasticity in hippocampal area CA1 of the rat. *J Neurophysiol* 107: 902–912, 2012. First published November 23, 2011; doi:10.1152/jn.00780.2011.—Long-term potentiation (LTP) is a form of synaptic plasticity thought to underlie memory; thus knowing its developmental profile is fundamental to understanding function. Like memory, LTP has multiple phases with distinct timing and mechanisms. The late phase of LTP (L-LTP), lasting longer than 3 h, is protein synthesis dependent and involves changes in the structure and content of dendritic spines, the major sites of excitatory synapses. In previous work, tetanic stimulation first produced L-LTP at postnatal day 15 (P15) in area CA1 of rat hippocampus. Here we used a more robust induction paradigm involving theta-burst stimulation (TBS) in acute slices and found the developmental onset of L-LTP to be 3 days earlier at P12. In contrast, at P8–11, TBS only reversed the synaptic depression that occurs from test-pulse stimulation in developing (P8–15) hippocampus. A second bout of TBS delivered 30–180 min later produced L-LTP at P10–11 but not at P8–9 and enhanced L-LTP at P12–15. Both the developmental onset and the enhanced L-LTP produced by repeated bouts of TBS were blocked by the *N*-methyl-D-aspartate receptor antagonist DL-2-amino-5-phosphonovaleric acid. Thus the developmental onset age is P12 for L-LTP induced by the more robust and perhaps more naturalistic TBS induction paradigm. Metaplasticity produced by repeated bouts of TBS is developmentally regulated, advancing the capacity for L-LTP from P12 to P10, but not to younger ages. Together these findings provide a new basis from which to investigate mechanisms that regulate the developmental onset of this important form of synaptic plasticity.

synaptic plasticity; learning; memory; ontogeny

LONG-TERM POTENTIATION (LTP) is an increase in synaptic efficacy following high-frequency stimulation that occurs in multiple brain regions and has long been considered a cellular mechanism for learning and memory (Bliss and Lomo 1973; Lynch et al. 1990; Madison and Schuman 1991; Bliss and Collingridge 1993; Bourne and Harris 2007). Induction paradigms for LTP have been refined since it was first discovered using 15-Hz stimulation in the hippocampus of anesthetized rabbit *in vivo* (Bliss and Lomo 1973). Tetanic stimulation, consisting of one or a few repetitions of 100 pulses delivered at 100 Hz, is one of the most commonly used paradigms to induce LTP. Prior work revealed that tetanic stimulation first produces L-LTP at postnatal day 15 (P15) in area CA1 of rat hippocampus (Harris and Teyler 1984; Jackson et al. 1993), consistent with subsequent studies (Muller et al. 1989; Bekenstein and Lothman 1991; Figurov et al. 1996). More recently, theta-burst

stimulation (TBS) has become recognized as a more robust LTP induction paradigm, especially in hippocampal area CA1 (Larson et al. 1986; Abraham and Huggett 1997). TBS resembles some of the normal patterns of hippocampal firing during development (Buzsaki et al. 1987; Nguyen and Kandel 1997; Morgan and Teyler 2001; Leinekugel et al. 2002; Buzsaki 2002; Mohns and Blumberg 2008), and it engages multiple mechanisms. In addition to the *N*-methyl-D-aspartate receptor (NMDA) receptor mechanisms induced by tetanic stimulation, TBS engages back-propagating action potentials (Johnston et al. 1999; Raymond 2008), activation of voltage-gated calcium channels (Ito et al. 1995), calcium release from internal stores (Raymond and Redman 2002; 2006), and release of brain-derived neurotrophic factor (BDNF) (Kang et al. 1997; Chen et al. 1999), all of which enhance LTP. Hence, we wanted to learn whether TBS might reveal an earlier developmental onset of L-LTP.

An important confounding variable in determining the developmental onset of LTP in hippocampus was discovered in recent work showing that delivery of baseline test pulses causes synaptic depression (Xiao et al. 2004; Abrahamsson et al. 2007; 2008). High-frequency stimulation reverses this depression, a phenomenon termed “developmental LTP” (Xiao et al. 2004; Abrahamsson et al. 2007; 2008). Because LTP is usually measured as an increase in synaptic responses relative to baseline responses acquired just before TBS, previous reports of LTP in the developing hippocampus had a component that constitutes reversal of test-pulse depression (Abrahamsson et al. 2007; 2008). Here analyses were performed relative to the initial “naïve” responses so that L-LTP was detected in addition to the de-depression of test-pulse stimulation. We established a new threshold age for L-LTP at P12. In mature animals, it is known that the production of LTP is regulated by previous synaptic activity, a form of synaptic plasticity known as “metaplasticity” (Abraham and Bear 1996). We showed for the first time that metaplasticity induced by multiple bouts of TBS shifts the developmental threshold for L-LTP to an even younger age at P10.

MATERIALS AND METHODS

Slice preparation. Procedures were approved by The University of Texas at Austin Institutional Animal Care and Use Committee and complied with all NIH requirements for the humane care and use of laboratory rats. Hippocampal slices were rapidly prepared from male Long-Evans rats at P8 to P35 as previously described (Bourne et al. 2007). Animals were decapitated using a guillotine; those older than P15 were first anesthetized with halothane. The brain was removed from the cranium, and the left hippocampus was dissected out and rinsed with room temperature artificial cerebrospinal fluid (aCSF) containing (in mM): 117 NaCl, 5.3 KCl, 26 NaHCO₃, 1 NaH₂PO₄, 2.5

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CaCl₂, 1.3 MgSO₄, and 10 glucose, pH 7.4, and bubbled with 95% O₂-5% CO₂. Four slices (400 μm thick) from the middle third of the hippocampus were cut at 70° transverse to the long axis on a tissue chopper (Stoelting, Wood Dale, IL) and transferred in aCSF to the supporting net in a single interface chamber (Fig. 1A) or to one of four interface chambers in the Synchronoslice system (Lohmann Research Equipment, Castrop-Rauxel, Germany; Fig. 2A). The entire dissection and slice preparation took ~5 min, crucial timing for L-LTP (Reymann and Frey 2007) and high-quality ultrastructure (Harris and Teyler 1984; Jensen and Harris 1989; Bourne et al. 2007).

Electrophysiology. Each chamber contained one hippocampal slice located on a net at the liquid-gas interface between 32–32.5°C aCSF, and humidified 95% O₂-5% CO₂ atmosphere bubbled through 35–36°C distilled water. After 3 h of incubation, stimulating and recording electrodes were positioned either within a single slice (Fig. 1A) or in the same location for each of four slices from the same animal (Fig. 2A). The rationale for these two experimental paradigms is discussed below in RESULTS and DISCUSSION.

For the within-slice experiments, two concentric bipolar stimulating electrodes (Fredrick Haer, Bowdoinham, ME) were placed in the middle of stratum radiatum at 400 μm to either side of a single recording electrode (Fig. 1A). In the multislice experiments, a concentric bipolar stimulating electrode was positioned near the CA3 side (S, Fig. 2B), and a metal recording electrode (platinum/tungsten core, impedance: ~0.5 MΩ; Thomas Recording, Geissen, Germany) was placed ~400 μm away from the stimulating electrode, also in the middle of CA1 stratum radiatum (R, Fig. 2B).

Stimuli consisted of 200-μs biphasic current, lasting 100 μs each for positive and negative components of the stimulus. Beginning 20 min after the electrodes were inserted, a stimulus-response curve was generated by measuring the slope (mV/ms) of the extracellular field excitatory postsynaptic potentials (fEPSPs) in response to increasing stimulus intensities (ranging from 100–400 μA). The fEPSP slopes (mV/ms) were estimated by linear regression during the initial phase of each evoked waveform. For the within-slice experiments, maximal fEPSP slope responses ranged from 1.3 to 5.2 mV/ms at P8–P9, 2.2 to 8.3 mV/ms at P10–P11, and 3.4 to 10.6 mV/ms at P12–P15. For the multislice experiments, the maximal slope responses ranged from 1.0 to 2.3 mV/ms at P8–P9, 1.1 to 4.2 mV/ms at P10–P11, 2.0 to 4.8 mV/ms at P12–P15, and 3.6 to 11.5 mV/ms at P19–35. The stimulus intensity required to obtain ~1/2 maximal fEPSP slope was held constant for the duration of each experiment for both the within-slice and multislice experiments. For the within-slice experiments, test pulses were alternated between the control and TBS-LTP electrode once every 2 min at a 30-s interval between electrodes. After completing rate-testing experiments (Fig. 3), we set the test-pulse rate to once every 5 min in the multislice paradigm.

For both types of experiments, TBS consisted of eight trains with 30-s intervals with each train containing 10 bursts at 5 Hz and each burst containing 4 pulses at 100 Hz (Fig. 2C) because this paradigm was previously shown in adult hippocampal area CA1 to produce saturating LTP (Abraham and Huggett 1997). A single bout of TBS was delivered at time zero for the within-slice experiments (green arrow, Fig. 1, B–I). Multiple paradigms of TBS stimulation (Fig. 1D) were tested in the multislice experiments as described in RESULTS. In the within-slice experiments, LTP was expressed as the percentage of baseline response obtained just 20 min before TBS, whereas, in the multislice experiments, LTP was expressed as a percentage of the first test pulse-induced fEPSP, called the “naïve response” as discussed below. For the 2-amino-5-phosphonovaleric acid (APV) experiments, 10 μl of a concentrated solution consisting of 10 mM DL-APV was added to two of the interface chambers giving a final concentration of 100 μM DL-APV 30 min before the first TBS (Fig. 7).

The stimulation and data acquisition were obtained using custom Igor software for the within-slice experiments and the Synchronoslice software (Lohmann Research Equipment) for the multislice experiments. It is worth noting that exact placement of stimulating and

recording electrodes can affect the fEPSP values; hence representative waveforms are illustrated, and average percentage responses (means ± SE) relative to pre-TBS stimulation are graphed.

Statistics. The STATISTICA package (StatSoft, Tulsa, OK) was used to calculate *P* values from overall ANOVAs followed by Tukey’s honestly significant difference (HSD) post hoc test.

RESULTS

Within-slice tests for developmental onset of L-LTP. The within-slice paradigm was tested first (Fig. 1A) because that was the paradigm originally used to discover that P15 is the developmental onset of L-LTP induced by tetanic stimulation (Harris and Teyler 1984; Jackson et al. 1993). A single bout of TBS (Fig. 1A) was delivered to one or the other of two independent pathways (stim 1 or stim 2) in alternating experiments at *time 0*, and the other electrode received control stimulation only (see MATERIALS AND METHODS). As in the past, potentiation was measured as a percent relative to the responses averaged across 20 min just before delivery of the TBS (i.e., percentage of baseline, Fig. 1). At P8–9, there was no significant potentiation relative to this prior baseline during the 4 h following TBS (Fig. 1, B and C). At P10–11, there was an initial potentiation that lasted about an hour and then decayed to baseline (Fig. 1, D and E). The TBS protocol first produced L-LTP at P12, which lasted the full 4 h of recording post-TBS (Fig. 1F). The endurance and level of L-LTP at P12 was similar to that produced at P13–15 (Fig. 1, G–I). ANOVA revealed an overall effect of age and time post-TBS ($F = 15.644$; $df = 23, 545$; $P < 0.001$, where *df* represents the degrees of freedom), and the post hoc analysis showed significant potentiation during the first hour beginning at P10; L-LTP measured across the third hour was significant beginning at P12 (Fig. 1J). Because of the long recording period required to test for L-LTP, only one within-slice experiment could be conducted per animal. In addition, response decrement to ongoing control stimulation, and in some cases following TBS, was obvious in the P8–14 slices (Fig. 1). Hence we developed a multislice system that allowed for simultaneous testing from four slices (Fig. 2), which made it possible to investigate test pulse-induced synaptic depression, multiple bouts of TBS separated by different times, and the role of NMDA receptor activation by blocking with the antagonist APV. These experiments are described next.

Age and frequency dependence of test pulse-induced synaptic depression. We first wanted to establish whether the test pulse-induced synaptic depression that was previously reported in developing animals (Xiao et al. 2004; Abrahamsson et al. 2007; 2008), and also observed in the within-slice experiments described above (Fig. 1), could be reduced at lower stimulation rates. Extracellular field potentials were recorded from area CA1 in stratum radiatum simultaneously in four slices from the same animal using the multislice system (Fig. 2, A and B). Four test-pulse frequencies commonly used in LTP experiments were investigated, including one pulse per 30 s (1 p/30 s), 1 pulse per 1 min (1 p/1 min), 1 p/2 min (Fig. 2Da), and 1 p/5 min (Fig. 2Db).

Relative to the first naïve test pulse, subsequent test pulses induced significant depression at all four frequencies at P8–12. The amount of depression was less at the 1 p/5 min rate and was significantly reduced relative to the faster rates by 4–5 h after the onset of stimulation (Fig. 3A). At P19–35, there was

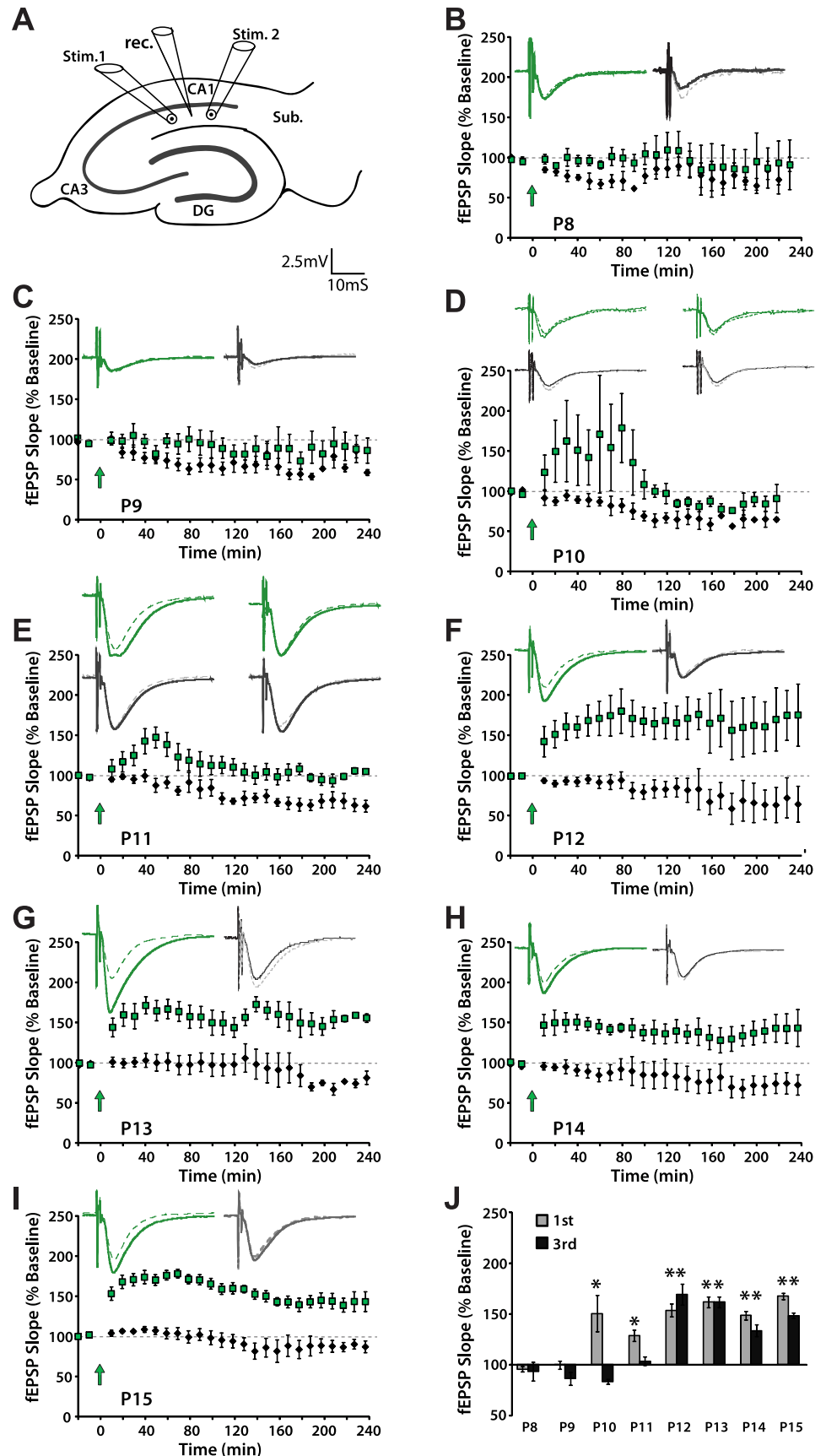


Fig. 1. Within-slice experiments. *A*: arrangement of stimulating (Stim. 1, 2) and recording (rec.) electrodes in the middle of stratum radiatum of hippocampal area CA1. DG, dentate gyrus. *B–I*: for all ages, the field excitatory postsynaptic potential (fEPSP) slope is expressed as a percentage of the average baseline response obtained for 20 min before delivery of theta-burst stimulation (TBS) at time 0 (green arrow) to 1 of the stimulating electrodes, alternating between locations across experiments at each age (see Fig. 2C for TBS pattern that was used). Responses following TBS are plotted in green (waveform and graph points), and control responses from the other stimulating electrode are plotted in black (waveforms and graph points). In *B*, *C*, *F*, *G*, *H*, *I*, the example waveforms represent a 20-min average of the pre-TBS responses, which are plotted as dotted lines of the matching color, and the 20-min average of the responses beginning 180 min post-TBS, which are plotted as solid lines. In *D* and *E*, the left most waveforms are comparisons between the pre-TBS 20-min period and at time 60 min, whereas the last set are comparisons from 180 min post-TBS. The scale bar in *A* is for all waveforms. *B* and *C*: no significant potentiation at postnatal day 8 (P8) ($n = 4$) or P9 ($n = 6$). *D* and *E*: short-lasting potentiation at P10 ($n = 7$) and P11 ($n = 7$). *F–I*: long-lasting potentiation at P12 ($n = 6$), P13 ($n = 6$), P14 ($n = 6$), and P15 ($n = 8$). *J*: comparison of responses at each age during the first hour (averaged from time 0–1 h) and third hour (averaged from time 3–4 h) following TBS relative to pre-TBS baseline (*post hoc $P < 0.05$).

no test pulse-induced depression at 1 p/5 min (Fig. 3*B*). ANOVAs were computed at each age and revealed significant overall effects at P8–9 ($F = 39.937$; $df = 5, 648$; $P < 0.001$), P10 ($F = 20.914$; $df = 5, 195$; $P < 0.001$), P11 ($F = 60.421$; $df = 5, 232$; $P < 0.001$), P12 ($F = 37.074$; $df = 5, 246$; $P < 0.001$), and P13–15 ($F = 16.425$; $df = 5, 232$; $P < 0.001$), but no significant effects at P19–35 were seen. The post hoc Tukey's HSD test was conducted at hourly intervals relative to

the first hour (-1 h) and revealed a statistically significant depression lasting the full 5 h at P8–15 and no significant depression for any time point at P19–35 (Fig. 3*C*). If the criterion for effective depression was set at -20% (the counterpart of $+20\%$ for effective LTP as discussed below), then the test-pulse depression lasted for less than 3 h at P12–15. Thus, to minimize test pulse-induced synaptic depression while still being able to obtain enough responses to track potentiation, we used the 1 p/5 min rate for test pulses in all subsequent experiments.

L-LTP produced by a single bout of TBS during development. We next wanted to determine whether the TBS induction protocol elicited L-LTP relative to naïve responses. TBS was applied 1 h after the start of test-pulse stimulation (1 p/5 min), and responses were then monitored at the same stimulation rate and intensity for 4.5 h to test for L-LTP (Fig. 2*Dc* and Fig. 4). At P8–11, TBS reversed the test-pulse depression, returning synaptic responses to the naïve level by 2–3 h post-TBS (Fig. 4, *A*, *B*, and *C*). At P12, TBS reversed test-pulse depression and produced L-LTP, which plateaued at $126.4 \pm 12.3\%$ above naïve responses (Fig. 4*D*), whereas, at P13–15, the magnitude of L-LTP was somewhat higher ($141.6 \pm 8.0\%$, Fig. 4*E*). The L-LTP achieved at P19–35 was markedly higher ($244.5 \pm 38.5\%$) than at younger ages (Fig. 4*F*), an effect that might have been influenced by the greater baseline response capacity in the older ages.

Metaplasticity of TBS-induced L-LTP during development. The experiments described above illustrate that TBS can reverse test pulse-induced depression at P8–11, but no L-LTP was produced relative to the initial naïve response. We wondered whether additional TBS might induce L-LTP at younger ages. In an adjacent slice from the same animals as those reported above, a second TBS was applied 90 min after the first TBS (T2 in Fig. 2*Dd* and T90T red arrows and plots in Fig. 5). At P8–9, the second TBS caused an initial depression (down from $75.4 \pm 4.4\%$ to $52.4 \pm 4.2\%$ of naïve level), and then the response gradually returned to the naïve level with no subsequent LTP (Fig. 5*A*). The second TBS also produced a small depression at P10 (down from $100.7 \pm 8.4\%$ to $87.3 \pm 7.4\%$ of naïve level) and at P11 (down from $89.8 \pm 6.4\%$ to $72.6 \pm 6.6\%$ of naïve level), which was reversed a few minutes later and resulted in L-LTP (Fig. 5, *B* and *C*). At P12, the second TBS also produced an initial depression (down from $104.6 \pm 5.1\%$ to $78.8 \pm 7.0\%$ of naïve level) followed by a return of

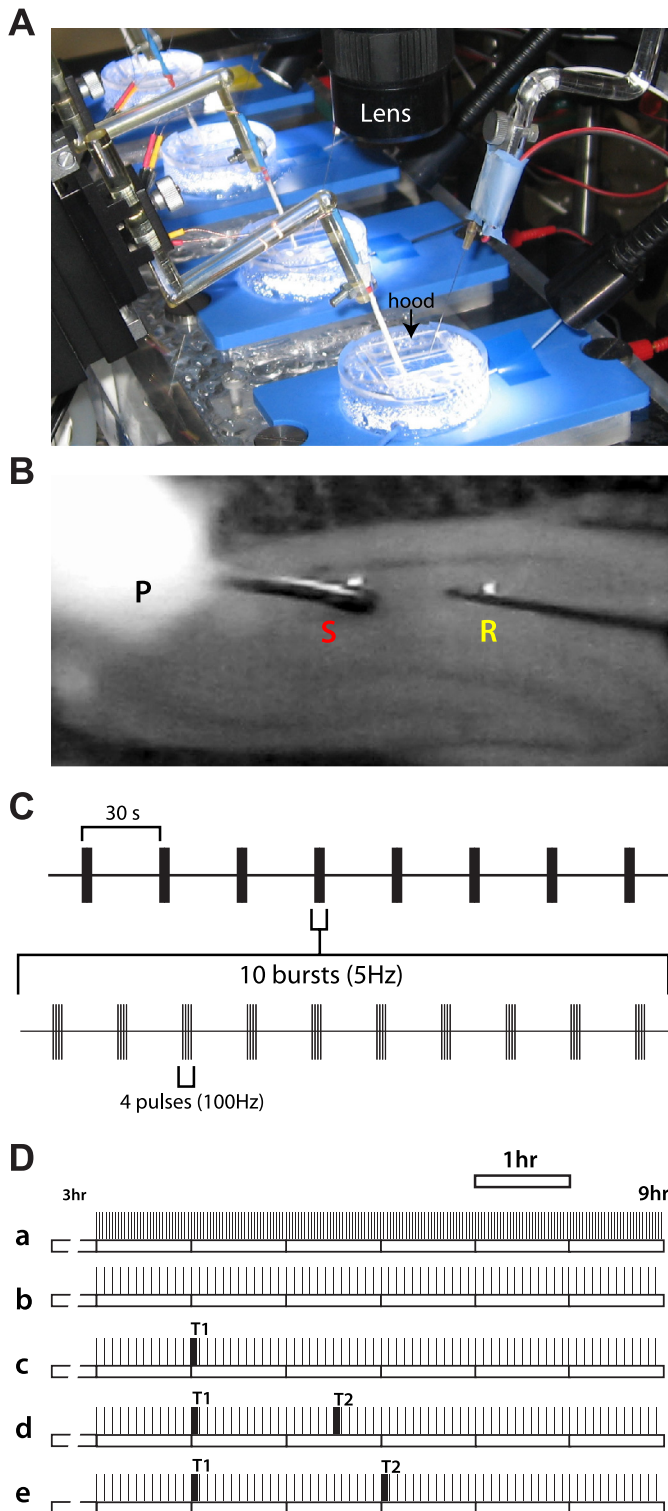


Fig. 2. Four-chamber multislice system. *A*: slices were viewed through 1 of the 4 camera lenses (Lens) located above each slice chamber, and electrodes were inserted into the slice through the sliding door on the top of the hood (arrow), which was custom designed to contain the humid and warm atmosphere (95% O_2 -5% CO_2). *B*: example hippocampal slice with stimulating (S) and recording (R) electrodes, which were positioned at the same locations in all 4 adjacent slices. Stimulating electrodes were wrapped with sleeves made of filter paper (P) to prevent condensation from dripping on the slices. *C*: each TBS comprised 8 trains delivered at 30-s intervals (*top*) with each train having 10 bursts delivered at 5 Hz and each burst having 4 pulses delivered at 100 Hz (*bottom*). *D*: stimulation protocols were independently controlled for each slice. Example stimulation paradigms are illustrated as follows: *a*: test pulses given at 1 pulse per 2 min (1 p/2 min); *b*: test pulses given at 1 p/5 min (see Fig. 3); *c*: one TBS (T1) applied at 1 h after the start of test-pulse stimulation given at 1 p/5 min (e.g., see Fig. 4); *d*: 2 TBS applied at 1 h (T1) and 2.5 h (T2) after the start of test-pulse stimulation given at 1 p/5 min (e.g., see Fig. 5); *e*: 2 TBS applied at 1 h and 3 h after the start of test-pulse stimulation given at 1 p/5 min (e.g., see Fig. 7).

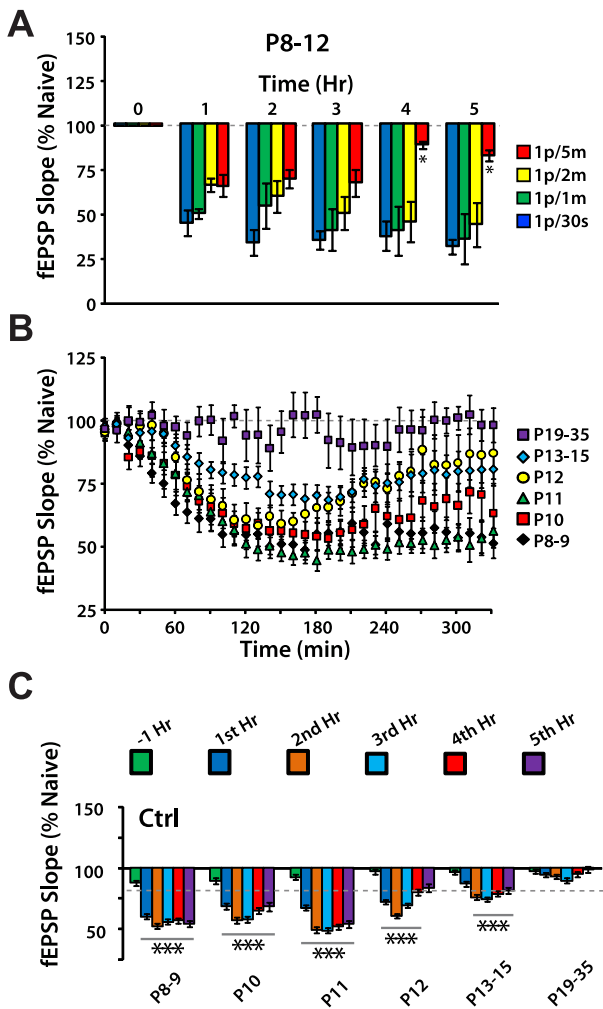


Fig. 3. Frequency and age dependence of synaptic depression induced by test-pulse stimulation. *A*: fEPSP slopes were normalized to the first naïve response at *time 0*. This graph illustrates that the synaptic depression induced by test pulses at different rates in animals aged P8–12 was significantly reduced at 1 p/5 min by 4–5 h ($n = 5$; $*P < 0.05$). *B*: response depression induced by test-pulse stimulation at 1 p/5 min in animals of different ages. (All data are expressed as means \pm SE across animals at each age and time point, with 1 slice per animal: P8–9, $n = 19$; P10, $n = 6$; P11, $n = 7$; P12, $n = 7$; P13–15, $n = 7$; P19–35, $n = 6$.) *C*: hourly analysis of age-dependent test pulse-induced depression at the stimulation rate of 1 p/5 min ($***P < 0.001$, dashed line at 80%).

L-LTP with a subtle further increase in magnitude ~ 2 h later (Fig. 5D). At P13–15, a second TBS enhanced the magnitude of L-LTP (Fig. 5E). At P19–35, no additional potentiation was detected following a second bout of TBS (Fig. 5F), consistent with prior reports from adult hippocampal area CA1 (Abraham and Huggett 1997). It is worth noting that the time course of the responses immediately following TBS was highly variable at the threshold ages (P8–P12). In some animals, and even across slices from the same animals, response recovery following the first TBS could require more than 90 min before reaching naïve baseline. In contrast, slices from other animals showed response recovery to baseline within a few minutes after the TBS.

Developmental regulation of metaplasticity advances the onset age of L-LTP. The onset age for L-LTP was determined by comparing fEPSP slope relative to the average response

during the first hour of test-pulse stimulation (-1 h), with criterion set at 120% magnitude and minimum duration of 3 h (Fig. 6A). Overall ANOVA revealed significant main effects across time at P12 ($F = 8.957$; $df = 5, 216$; $P < 0.001$), P13–15 ($F = 24.629$; $df = 5, 222$; $P < 0.001$), and P19–35 ($F = 34.210$; $df = 5, 149$; $P < 0.001$). Subsequent Tukey's HSD post hoc analyses confirmed significant and effective L-LTP (120%) lasting beyond 3 h at P12–35, but not at younger ages (Fig. 6A). Thus L-LTP was first elicited by a single bout of TBS at P12 and showed substantial enhancement as the animals matured.

We next tested whether the T90T protocol produced significantly more effective L-LTP than the T protocol (Fig. 6B). The overall ANOVA comparing LTP magnitude following a single T vs. T90T revealed significant main effects across time at P10 ($F = 23.276$; $df = 11, 345$; $P < 0.001$), P11 ($F = 85.23$; $df = 11, 375$; $P < 0.001$), P12 ($F = 26.556$; $df = 11, 530$; $P < 0.001$), and P13–15 ($F = 28.101$; $df = 11, 472$; $P < 0.001$). The Tukey's HSD post hoc analysis showed that L-LTP was produced at the younger ages of P10 and P11 following the T90T protocol. Furthermore, L-LTP compared during the fourth and fifth hours was enhanced at P12 and P13–15 in the T90T vs. the T protocol. Similar to what has previously been reported in adult hippocampus (Abraham and Huggett 1997), the first bout of TBS produced saturating L-LTP at P19–35 such that the magnitude of L-LTP did not differ significantly during the third to fifth hours between the T and T90T protocols. Thus the T90T protocol advanced the onset age of L-LTP from P12 to P10.

APV blocks TBS-induced L-LTP at P10–12. Many mechanisms might account for a second TBS producing L-LTP at P10–11 when a single TBS only reversed the synaptic depression. Here we tested whether activation of the NMDA receptor was involved. Two new sets of experiments were conducted in the multislice system in which $100 \mu\text{M}$ DL-APV was added 30 min before TBS (Fig. 7). Two slices received control stimulation at 1 p/5 min in the absence or presence of APV, which as expected had no effect on the synaptic-response depression (Fig. 7). In two experimental slices, the T90T protocol was delivered in the absence or presence of APV. The aforementioned variation in response recovery after the first TBS also occurred in these slices; nevertheless, the presence of APV always prevented production of L-LTP following the T90T protocol at P10–11 (Fig. 7A) and P12 (Fig. 7B).

Altering the interval between multiple TBS does not produce L-LTP at P8–9. Finally, we explored the time interval between multiple bouts of TBS to test whether earlier or later delivery might produce L-LTP at P8–9 or enhance L-LTP at P10–11 (Fig. 7). At P8–9, we tested time intervals of 30, 90, or 180 min following the first TBS. Interestingly, when the second TBS was delivered 30 min after the first at these young ages, it appears that the test pulse-induced depression did not recover to naïve baseline, suggesting perhaps that a form of long-term depression was induced (Fig. 7A, T30T). In contrast, when the second TBS was delivered at 90 or 180 min after the first TBS, the test-pulse depression was reversed, but no L-LTP ensued (Fig. 7A, T90T, T180T). These findings might reflect a difference in the susceptibility of DL- α -amino-3-hydroxy-5-methylisoxazole-propionic acid (AMPA) receptor trafficking at these

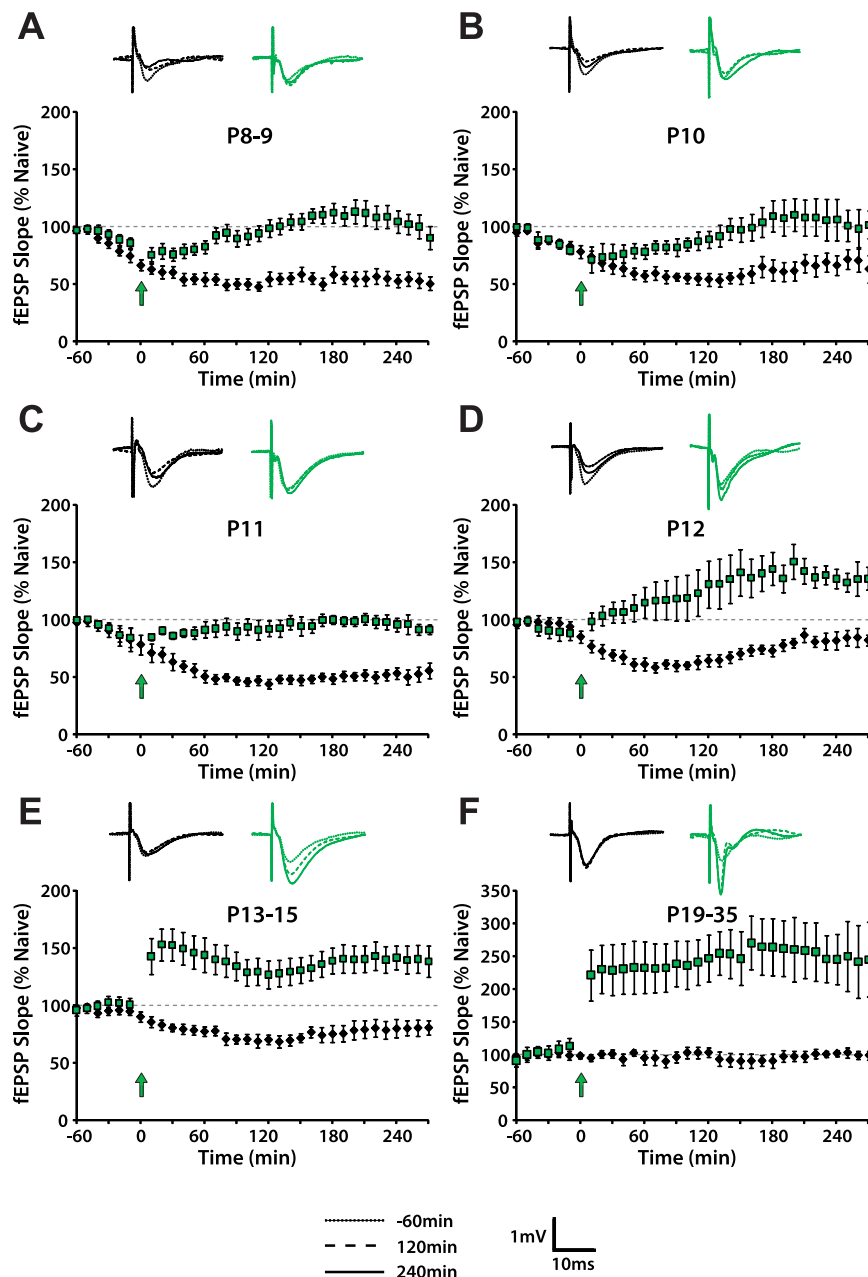


Fig. 4. TBS first produced late phase of long-term potentiation (L-LTP) at P12. TBS (delivered at *time 0*, green arrow) only reversed the test pulse-induced depression at P8–9 ($n = 12$) (A), P10 ($n = 6$) (B), and P11 ($n = 7$) (C). D: at P12, TBS produced slow-onset L-LTP, which plateaued at $\sim 40\%$ between 2–3 h after TBS ($n = 7$). E: at P13–15, LTP onset was immediate, resulting in L-LTP ($n = 6$). F: similarly, at P19–35 ($n = 6$), L-LTP had a fast onset, and the magnitude was nearly twice that attained at P12–15. The fEPSP slopes were normalized relative to the first naive response and then averaged across experiments and expressed as means \pm SE for each age. Legend is for the example waveforms displayed in each graph, where waveforms are displayed for control (black) and TBS (green) conditions at -60 , 120 , and 240 min relative to TBS, with scale bars at 1 mV per 10 ms. Similarly, the black graph points represent average (means \pm SE) control responses, and the green graph points are for average responses (means \pm SE) before and after TBS.

young synapses, which underlies test pulse-induced depression (Abrahamsson et al. 2007; 2008).

At P10–11, we found robust L-LTP when the second bout of TBS was delivered at 60, 90, or 120 min after the first TBS (Fig. 8B). Thus, at P10–11 but not at P8–9, synapses appear to have been primed by the first TBS such that a second TBS resulted in L-LTP. These findings suggest that P10 is the first age that TBS produces metaplasticity resulting in subsequent L-LTP.

DISCUSSION

In the present studies, we found that baseline synaptic depression was less when test pulses were delivered at 1 p/5 min than at higher frequencies; furthermore, test pulse-induced synaptic depression diminished with age and time in vitro. A single bout of TBS reversed test pulse-induced synaptic depression at P8–11 and first produced L-LTP at P12, and the

magnitude of L-LTP was enhanced as animals matured. A second TBS could produce L-LTP at P10–11 but not at younger ages. This effect was blocked by APV, suggesting that NMDA receptor activation was required at least during the second TBS to produce L-LTP at both P10–11 and P12. These findings provide a novel basis to test for shifts in the molecular, cellular, and synaptic properties of developing neurons needed to sustain L-LTP and the developmental onset of hippocampal-dependent learning and memory.

Test pulse-induced synaptic depression. In previous reports, test pulses were given at 1 pulse per 1, 5, or 20 s, and all of these higher frequencies showed the same amount of test pulse-induced synaptic depression, which was attributed to AMPA receptor “silencing” (Xiao et al. 2004; Abrahamsson et al. 2007; 2008). We found similar amounts of test pulse-induced synaptic depression at 1 p/30 s and 1 p/1 min, sug-

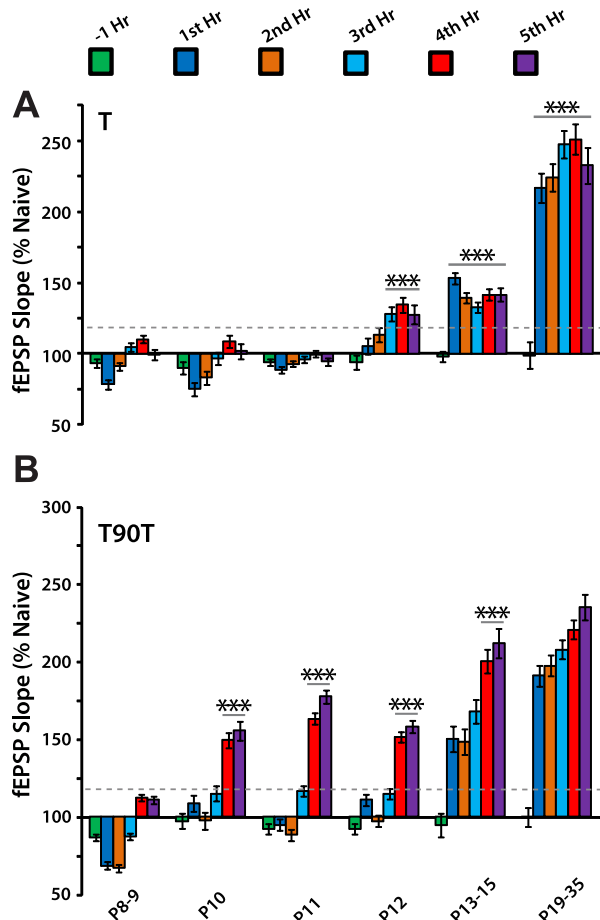


Fig. 6. Summary analyses comparing the developmental onset of L-LTP in response to 1 or 2 bouts of TBS. *A*: L-LTP onset age is P12 in response to a single TBS (T). *B*: metaplasticity of L-LTP following a second TBS in the T90T interval reveals significant L-LTP at P10 and P11, and greater LTP at P12–15 in the T90T interval relative to the T interval shown in *A*. (The dashed gray line is 120%, $***P < 0.001$.)

opmental LTP” at ages younger than P12 (Liao et al. 1995; Liao and Malinow 1996; Abrahamsson et al. 2008). Others have also reported that coincident tetanic stimulation of multiple inputs first induced developmental E-LTP at P13; however, the responses were only monitored for 30 min (Abrahamsson et al. 2008), and hence these studies would have missed the P12 onset age for L-LTP reported here. For both of our paradigms, there was variation in the time course of response recovery during the 90 min immediately following the first TBS at P8–P12. This variation might be due to the birthdate being set when pups were first observed, but, because pups are

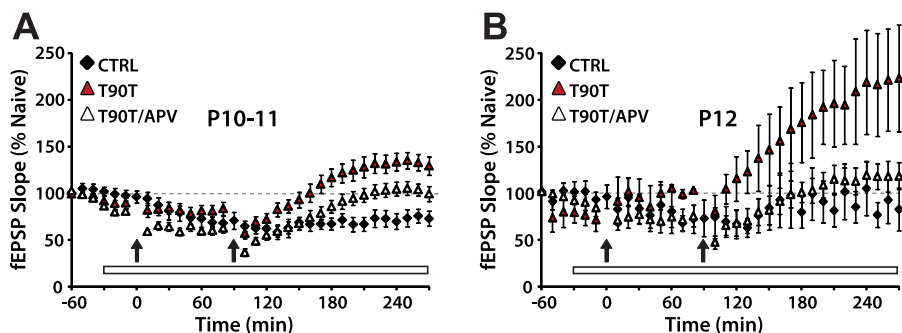


Fig. 7. 2-Amino-5-phosphonovaleric acid (APV) blocked the L-LTP normally produced by 2 TBS delivered at *time 0* and 90 min (black arrows) at P10–11 ($n = 8$) (*A*) and P12 ($n = 4$) (*B*). Legends: APV was added to the interface chambers at *time -30* min and remained throughout the experiments (horizontal bar) to ensure stability in electrode positioning following TBS. Control (CTRL) slices showed the same level of synaptic depression in the presence of APV as those without APV (data not shown for clarity). T90T in the absence of APV (red triangles) and T90T in the presence of APV (white triangles).

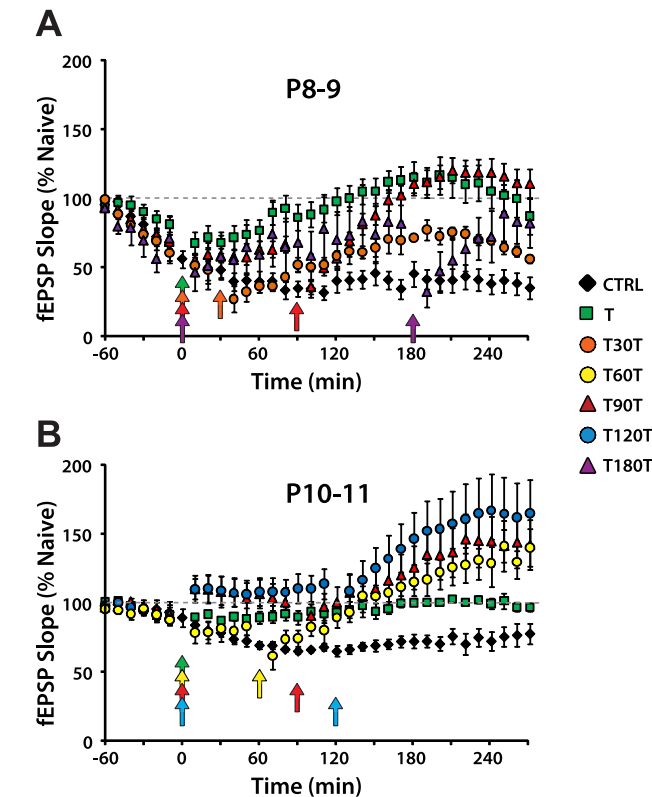


Fig. 8. Testing capacity of multiple TBS to produce L-LTP at younger ages. *A*: at P8–9, a second TBS was given 30 min (T30T orange, $n = 4$), 90 min (T90T red, $n = 20$), or 180 min (T180T purple, $n = 4$) after the first TBS; in each case test-pulse depression was reversed, but no L-LTP was produced. *B*: at P10–11, intervals of 60 min (T60T yellow, $n = 4$), 90 min (T90T red, $n = 12$), or 120 min (T120T blue, $n = 5$) all resulted in metaplasticity that produced L-LTP following the second TBS. In both graphs, average data from adjacent slices that were given a single TBS (T, green) or test pulses only (CTRL, black) are plotted for comparison.

often born at night, the exact date might be off by 12 h. Furthermore, although we obtained all slices from the middle third of the hippocampus, there might have been a developmental gradient along the septal temporal axis even within the middle third of these young animals that could have influenced the “relative age” of a particular slice. Thus, although the onset age for L-LTP from one bout of TBS occurred abruptly at P12 and the metaplasticity of two bouts of TBS started at P10, the timing of recovery from synaptic depression was less predictable.

There has been a strong link between the presence of dendritic spines and lasting LTP. Dendritic spines amplify voltage and compartmentalize calcium and other signaling

molecules near synapses, which are features thought to be necessary for LTP. In further support of the hypothesis that dendritic spines are a crucial component of L-LTP, molecular and live imaging approaches have shown coincident alterations in spine and synapse structure during LTP, largely through the modulation of actin filaments, scaffolding proteins, receptors, and other growth-promoting or -reducing factors at the synapse (Bonhoeffer and Yuste 2002; Ouyang et al. 2005; Alvarez et al. 2007; Sfakianos et al. 2007; Nagerl et al. 2007; Bourne and Harris 2008; Steiner et al. 2008; Loebrich and Nedivi 2009; Budnik and Salinas 2011). Dendritic spines are absent at P1–7, but it is not known when they first appear because no serial-section electron microscopy data exist from P8 to P11 (Fiala et al. 1998). Confocal microscopy suggests that a few dendritic spines might be present at P10–P11 (Kirov et al. 2004). Because tetanic stimulation did not produce enduring L-LTP at P12, it had been surprising to find some mature dendritic spines in developing rat hippocampus at P12 (Harris et al. 1989; Fiala et al. 1998; Kirov et al. 2004). The younger onset age at P12 for L-LTP following TBS provides consistent support for the spine hypothesis as a basis for the developmental onset of L-LTP at P12. It will be of interest to learn whether spines are present or produced by the first bout of TBS to support the developmental shift in expression of L-LTP from P12 to P10.

Dendritic and synaptic structure vary with ion channel and receptor density, which are also developmentally regulated (Maletic-Savatic et al. 1995; Kang et al. 1996; Miyashita and Kubo 1997; Hsia et al. 1998; Petralia et al. 1999; Rongo and Kaplan 1999; Sans et al. 2000; Molnar et al. 2002; Bender et al. 2007). For example, the NMDA receptor subunit composition is developmentally regulated with NR2B being gradually replaced by NR2A during the first two postnatal weeks (Takai et al. 2003). The NR3A subunit affects synaptogenesis, synapse maturation, and dendritic spine density (Das et al. 1998; Roberts et al. 2009), and NR3A density peaks at P8 and then gradually decreases after P12 (Wong et al. 2002). NMDA receptor activation is stronger at P7–9 than at P12–15 (Muller et al. 1989), and depolarization of young (P4) CA1 cells during synaptic activation produces E-LTP (Liao and Malinow 1996). Furthermore, we show that APV blocked L-LTP in the T90T paradigm at P10–P12. Thus it appears that NMDA receptors are not the limiting component for establishing the onset age for producing L-LTP by TBS.

Other factors align somewhat more closely with the L-LTP onset age. For example, the density of voltage-gated calcium channel increases with age and dendritic calcium influx after TBS is much greater at P12–14 than at younger ages (Isomura and Kato 1999). BDNF promotes neuronal survival and differentiation, and its expression is modified by neuronal activity (Patterson et al. 1992; Springer et al. 1994). Exogenous BDNF treatment has been shown to enhance production of L-LTP by tetanic stimulation in P12–13 but not P8–9 hippocampal slices (Figurov et al. 1996). BDNF infusion alone can induce LTP, whereas gene knockouts of BDNF or its TrkB receptors impair LTP (Minichiello et al. 1999; Bartoletti et al. 2002). BDNF mRNA is first detected at embryonic *day* 19, and it increases gradually and reaches the peak at P15 in rat brain (Ernfors et al. 1990; Kaisho et al. 1991). Perhaps BDNF released during the first TBS caused synaptogenesis, synapse maturation, and/or spine formation in support of subsequent L-LTP.

The extracellular space in hippocampal CA1 neuropil, which is abundant at P1–7, becomes substantially reduced by P12 as dendritic spines, axons, and dendrites increase and crowd the neuropil (Fiala et al. 1998). Astroglial processes, which provide critical factors for synapse maturation, begin to infiltrate the neuropil by P12 (Ullian et al. 2001; 2004; Stevens et al. 2007). The decreased extracellular space would help to concentrate glutamate and other neurotransmitters, as well as the astroglial factors, possibly facilitating spine and synapse maturation and production of L-LTP.

In apparent contradiction of a necessary role for spines in L-LTP, nonspiny dendrites of interneurons can also concentrate calcium in small dendritic domains and produce synaptic potentiation (Goldberg et al. 2003; Soler-Llavina and Sabatini 2006). Nonspiny interneurons are sparsely distributed; hence, intracellular recordings are needed to assess their plasticity. These recordings can only be held for about an hour, too short to assay L-LTP. Thus, although mechanisms are available to elicit short-lasting synaptic potentiation and E-LTP in the absence of dendritic spines, these have not been proven to produce L-LTP either on spine-free interneurons or early during development on essentially spine-free pyramidal cell dendrites, as shown here.

Enhancement of L-LTP with maturation. Recent experiments and computational models suggest that dendritic segments might be “minimal units” of synaptic plasticity, which share molecular and subcellular resources (e.g., calcium, enzymes, mRNA, etc.) between neighboring dendritic spines (Poirazi et al. 2003; Losonczy and Magee 2006; Govindarajan et al. 2006; 2011; Harvey et al. 2008). In addition, dendritic spines begin to acquire a spine apparatus sometime around P15. The spine apparatus is an organelle composed of smooth endoplasmic reticulum interdigitating with a densely stained material comprised of synaptopodin, an actin-binding protein (Deller et al. 2007). The spine apparatus is thought to regulate intraspine calcium as well as posttranslational modification and transport of locally synthesized proteins (Jedlicka et al. 2008). In the mature rat hippocampus (>P50), ~25% of all spines contain a spine apparatus, whereas nearly 80% of the largest mushroom-shaped spines contain a spine apparatus (Spacek and Harris 1997). Synaptopodin knockout mice have no spine apparatus and have impaired LTP, suggesting that the spine apparatus may serve to enhance LTP in older animals. Together, these findings suggest that, as dendrites become spiner with maturation, the opportunity to share plasticity resources between neighboring spines along shorter segments of dendrites increases, which may explain why we found the magnitude of L-LTP to be enhanced in the older animals.

Metaplasticity and advancing the developmental onset age of L-LTP. At P10–11, one TBS did not induce L-LTP, but two bouts of TBS were effective at multiple time intervals. This metaplasticity provides a direct basis from which to determine necessary components for production of L-LTP. Actin polymerization is critical for spine morphological changes after the induction of LTP, and the TBS paradigm induces actin polymerization (Lin et al. 2005). Dendritic filopodia have a high concentration of actin, raising the question of whether existing dendritic filopodia were encouraged by the first TBS to transition into dendritic spines, as has been seen both spontaneously and in response to synaptic activation in cultured hippocampal neurons (Dailey and Smith 1996; Maletic-Savatic

et al. 1999; Nagerl et al. 2007). Did promotion of filopodia to spines allow synapses on them to experience sufficient voltage amplification, receptor activation, ion channel gating, and concentration of calcium and other key molecules in spine heads that are needed to produce L-LTP during the second TBS? Did the priming TBS engage new protein synthesis locally to support subsequent L-LTP? This developmental regulation of metaplasticity provides an exciting opportunity to determine which components are essential for the natural expression of L-LTP during development and should provide new insight into mechanisms that are necessary for the normal development of learning and memory.

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DISCLOSURES

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