Cellular/Molecular

Persistent but Labile Synaptic Plasticity at Excitatory Synapses

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Short-term synaptic plasticity contributes to many computations in the brain and allows synapses to keep a finite record of recent activity. Here we have investigated the mechanisms underlying an intriguing form of short-term plasticity termed labile LTP, at hippocampal and PFC synapses in male rats and male and female mice. In the hippocampus, labile LTP is triggered by high-frequency activation of presynaptic axons and is rapidly discharged with further activation of those axons. However, if the synapses are quiescent, they remain potentiated until further presynaptic activation. To distinguish labile LTP from NMDAR-dependent forms of potentiation, we blocked NMDARs in all experiments. Labile LTP was synapse-specific and was accompanied by a decreased paired pulse ratio, consistent with an increased release probability. Presynaptic Ca^{2+} and protein kinase activation during the tetanus appeared to be required for its initiation. Labile LTP was not reversed by a PKC inhibitor and did not require either RIM1α or synaptotagmin-7, proteins implicated in other forms of presynaptic short-term plasticity. Similar NMDAR-independent potentiation could be elicited at synapses in mPFC. Labile LTP allows for rapid information storage that is erased under controlled circumstances and could have a role in a variety of hippocampal and prefrontal cortical computations related to short-term memory.

Key words: hippocampus; LTP; memory; PFC; synaptic plasticity; working memory

Significance Statement

Changes in synaptic strength are thought to represent information storage relevant to particular nervous system tasks. A single synapse can exhibit multiple overlapping forms of plasticity that shape information transfer from presynaptic to postsynaptic neurons. Here we investigate the mechanisms underlying labile LTP, an NMDAR-independent form of plasticity induced at hippocampal synapses. The potentiation is maintained for long periods as long as the synapses are infrequently active, but with regular activation, the synapses are depotentiated. Similar NMDAR-independent potentiation can also be induced at L2/3-to-L5 synapses in mPFC. Labile LTP requires a rise in presynaptic Ca^{2+} and protein kinase activation but is unaffected in RIM1α or synaptotagmin-7 mutant mice. Labile LTP may contribute to short-term or working memory in hippocampus and mPFC.

Introduction

Changes in synaptic strength are thought to represent information storage relevant to particular nervous system tasks. Evidence accumulated over many years supports an important role for NMDAR-dependent LTP in learning and memory, as well as in other neuroadaptations to external stimuli (brain development, addiction, nociception) (Malenka and Bear, 2004; Nicoll, 2017). Perhaps the most widely studied synapse in the vertebrate brain is that between hippocampal CA3 and CA1 pyramidal cells. Specific deletion of NMDARs at these synapses produces deficits in spatial learning tasks and disruption of place fields (Bannerman et al., 1995; Tsien et al., 1996; Nakazawa et al., 2004; Place et al., 2012; Morris, 2013; Morris et al., 2013).

A single synapse can exhibit multiple overlapping forms of plasticity that shape information transfer from presynaptic to postsynaptic neuron. Short-term plasticity occurs at nearly all synapses, even after as few as two presynaptic action potentials. Facilitation, short-term depression, endocannabinoid-mediated

Received Sept. 25, 2017; revised April 11, 2018; accepted April 19, 2018.

Author contributions: B.P., K.L., K.T.T., and J.A.K. designed research; B.P., K.L., K.T.T., C.J.F., M.A.N., and J.A.K. performed research; B.P., K.L., K.T.T., C.J.F., M.A.N., and J.A.K. analyzed data; B.P. and J.A.K. wrote the paper.

This work was supported by National Institutes of Health Grants NS050570 and NS088453 to J.A.K., Brown Institute for Brain Science to B.P., and a Deutsche Forschungsgemeinschaft Grant PR1719/1-1 to B.P. We thank Rachel Stevenson, other J.A.K. laboratory members, and Drs. Carlos Aizenman, Barry Connors, Chris Moore, David Badre, and Theresa Desrochers for helpful suggestions; and Ayumi Tsuda for technical assistance.

The authors declare no competing financial interests.

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DOI:10.1523/JNEUROSCI.2772-17.2018
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short-term depression, and post-tetanic potentiation (PTP) modulate responses based on the recent history of activation of those synapses (Zucker and Regehr, 2002; Chevaleyre et al., 2006; Fioravante and Regehr, 2011; Jackman and Regehr, 2017), and specific synapses often have a characteristic short-term plasticity signature (e.g., rapidly developing depression during repeated action potentials). On a short time scale, many cognitive tasks require that a neural ensemble be persistently active or primed to retain an item of information on a seconds to tens of seconds time scale. Pattern completion, maintenance of temporal order, and working memory all require information to be retained in response to transient sensory input (Funahashi et al., 1989; Goldman-Rakic, 1995; Abbott and Regehr, 2004; Major and Tank, 2004; Kamiński et al., 2017; Wolff et al., 2017). It has been theorized that this is achieved by persistent firing of relevant neurons, but recent work in rodents and humans suggests that synaptic potentiation may also contribute in an "activity-silent" manner (Funahashi et al., 1989; Barak and Tsodyks, 2007; Hansel and Mato, 2013; Wolff et al., 2015; Rose et al., 2016).

CA3-to-CA1 hippocampal synapses exhibit short-term facilitation, PTP, endocannabinoid depression, and NMDAR-dependent LTP (Ohno-Shosaku et al., 2002; Fioravante and Regehr, 2011; Herring and Nicoll, 2016). At the same synapses, we have now characterized a form of synaptic potentiation that is initiated by high-frequency activity of CA3 pyramidal cell axons and is apparently stored for seconds, or for up to hours as long as the synapse is stimulated infrequently. However, the synapses are depotentiated when activation at higher frequency is resumed. This plasticity, which has been called transient LTP or labile LTP, has been noted previously (Xiao et al., 1996; Volianskis and Jensen, 2003), but is often not distinguished in LTP experiments because typical test stimulus frequency causes relatively rapid depotentiation. Labile LTP has properties that might be useful in retaining information for short periods, but also allows synaptic strength to reset rapidly after use. Here we have probed the frequency dependence, synapse specificity, and molecular and pharmacological properties of labile LTP.

Materials and Methods

Slice preparation. All experiments were performed strictly adhering to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and as approved by the Brown Institutional Animal Care and Use Committee. Male Sprague Dawley rats were used; in some experiments, RIM1α knock-out mice (JAX B6;129P2Rims1^{tm1Sud}/J) and synaptotagmin-7 (syt-7) knock-out mice (JAX B6.129.S1-Syt7^{tm1Nan}/J) were used. RIM1α knock-out mice were bred as heterozygotes crossed to heterozygotes, syt-7 knock-out mice were bred as homozygotes to homozygotes, and homozygote results are reported here. Each strain had been backcrossed onto a C57BL/6J background for at least 7 generations. Both male and female mice were used. Mice (p28–40) or male Sprague Dawley rats (Charles River, p28–40), were deeply anesthetized with isoflurane and decapitated. Coronal brain slices were prepared as described previously (Kauer, 1999; Mair and Kauer, 2007). The 400-μm-thick slices (300 μm for whole-cell recordings) were placed into ice-cold ACSF containing the following (in mM): 126 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.3 NaH_2PO_4, 2.5 $CaCl_2$, 1.2 $MgSO_4$, or 124 NaCl, 3.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 2 $MgSO_4$, 26 $NaHCO_3$, saturated with 95% O_2-5%CO_2 (Volianskis and Jensen, 2003). No differences were observed in the two ACSF compositions. For prefrontal cortical slices, two slices per animal ~200–300 μm caudal to the olfactory bulb and just rostral to the basal ganglia were prepared (plates 8–10, Paxinos and Watson, 2013). Slices were stored for 1–6 h submerged in oxygenated ACSF at room temperature.

Electrophysiological recordings. Slices were placed in a submerged recording chamber constantly perfused at ~2–3 ml/min with bubbled ACSF and heated to 29°C–31°C. In all experiments, 50 μM D-APV was included in the ACSF to block NMDARs. Field potentials were evoked and recorded from area CA1 as described previously (Kauer, 1999); and in mPFC slices, the stimulating electrode was placed in layer 2/3 and the recording electrode in layer 5 (Mair and Kauer, 2007). The recording electrode was filled with 2N NaCl and placed in stratum radiatum. In experiments for Figure 2, stimulating electrodes were positioned on either side of the recording electrode (all in stratum radiatum), and stimulation was delivered alternately to each input. Paired pulses were evoked at 50 ms interstimulus interval. Field potentials were recorded with an Axopatch or AM Systems amplifier and Brownlee postamplifier and filtered at 3 kHz for storage on a PC. The initial slope of the field potential was measured with custom software written for Labview and kindly donated by Dr. Daniel Madison. Amplitudes rather than slopes of mPFC fields were measured because of their rapid time course. Baseline stimulation frequency was 1/45 s (0.022 Hz) except as noted. High-frequency stimulation (HFS) was delivered at 100 Hz for 1 s at 1.5 times the test current intensity; this train was repeated twice, 20 s apart except for data in Figure 2 in which the train was repeated 4 times. After HFS in most experiments, stimulation was not resumed for at least 5 min and was then resumed at once per 5 min (0.00334 Hz) except as noted.

For extracellular EGTA-AM experiments, slices were either incubated for 1 h before and during recording in 200 μM EGTA-AM or 200 μM EGTA-AM was added to the bath solution after recording began. For intracellular EGTA experiments, whole-cell recordings were made from visually identified CA1 pyramidal cells as described previously (Gibson et al., 2008). The recording solution was identical to ACSF except that 50 μM picrotoxin was included to block $GABA_A$ receptors. Patch pipettes were filled with internal recording solution containing the following (in mM): 117 K gluconate, 2.8 NaCl, 5 $MgCl_2$, 20 HEPES, 2 ATP-Na^+, 0.3 GTP-Na^+, and 15 EGTA. Neurons were held at −70 mV throughout the recording period, and for at least 20 min before HFS to allow EGTA to diffuse to synaptic sites, a protocol known to attenuate various forms of LTP dependent upon postsynaptic Ca^{2+} (Lenz and Alger, 1999; Cho et al., 2001; Lapointe et al., 2004). The cell input resistance and series resistance were monitored throughout each experiment; cells were discarded if these values changed by >10% during the experiment. Recordings were amplified and low-pass filtered at 3 kHz and digitally sampled using pCLAMP software (Molecular Devices). EPSC amplitudes were measured using pClamp.

Experimental design and statistical analysis. Because of the low stimulation frequencies, paired pulse ratios (PPRs) were calculated for each time point as slope fEPSP2/fEPSC1 (hippocampus), fEPSP2/fEPSP1 (PFC), or amplitude EPSC2/EPSC1. All results are expressed as the mean ± SEM. The slope or amplitude of the average of 15 fEPSPs/EPSCs before HFS was compared with all fEPSPs/EPSCs recorded after tetanus for statistical testing (7–14 points), unless otherwise noted. For Figure 2 experiments using alternately stimulated pathways, experiments in which the untetanized pathway potentiated >10% were excluded to demonstrate that even when no significant potentiation occurs on the untetanized pathway, significant labile LTP is still observed. Statistical significance was determined using paired *t* tests on raw data before and after a manipulation except where noted, and *p* values <0.05 were considered significant and are denoted with an asterisk. *N* values were based on previous experience and represent the number of slices used, except for experiments with knock-out mice for which *n* equals number of mice. Experiments using RIM1α knock-out mice were performed blind to genotype; CA3-CA1 synapses from syt-7 mice lack paired pulse facilitation, making blinding imperfect.

Materials. GF109203X (Tocris Bioscience) and EGTA-AM (Invitrogen) were dissolved in DMSO and diluted 1:1000 for use; 0.1% DMSO had no effect on labile LTP (data not shown). All other reagents were from Sigma or Tocris Bioscience and were dissolved in water or ACSF.

Results

HFS of CA3 afferents in the hippocampal slice is followed by transient potentiation (labile LTP) (Volianskis and Jensen, 2003). Although NMDAR antagonists were reported to prevent labile

LTP induction (Volianskis and Jensen, 2003; Volianskis et al., 2013b, 2015), we find instead that, even with NMDARs blocked using 50 μM D-APV, robust labile LTP was induced (Fig. 1). This D-APV-resistant transient potentiation was still observed even with a pause in stimulation after HFS for 10–20 min, a time point when short-term facilitation, depression, and PTP have recovered to control levels, confirming previous observations (Volianskis and Jensen, 2003). The onset and subsequent decrement of labile LTP after tetanus were paralleled by a decreased PPR that gradually returned to baseline values, consistent with the idea that labile LTP results from a transient increase in presynaptic transmitter release (compare Volianskis and Jensen, 2003). Blocking NMDARs distinguishes labile LTP from common forms of LTP and NMDAR-dependent short-term potentiation (Collingridge et al., 1983; Kauer et al., 1988; Malenka, 1991; Park et al., 2014). All of the subsequent experiments were performed in the presence of 50 μM D-APV to isolate NMDAR-independent synaptic plasticity.

After the tetanus, if the stimulation frequency was reduced to a very low rate (once per 5 min), labile LTP was maintained, no longer exhibiting any decrement over periods of ≥50 min after tetanus (Fig. 2*a*). Again, the potentiation was accompanied by a significant drop in the PPR. Control pathways recorded simultaneously in the same slices did not exhibit these changes in EPSP slope or PPR (Fig. 2*b*), demonstrating that labile LTP is synapse-specific, apparently not induced at neighboring synapses that do not receive HFS. Together, these data suggest that brief repetitive activation of presynaptic afferents allows these activated synapses to retain information for long periods, but only if the synapses are activated infrequently after the triggering event.

Do other excitatory synapses with a likely role in memory formation also exhibit labile LTP? We next tested excitatory synapses in slices of mPFC, a brain region strongly implicated in working memory. In the presence of D-APV, fEPSPs recorded in layer 5 while stimulating layer 2–3 exhibited modest potentiation 2 min after HFS when stimulated at 0.1 Hz (Fig. 3*a–c*). If instead, these synapses were stimulated infrequently 10 min after HFS, they remained potentiated (Fig. 3*d–f*). Similarly to hippocampal synapses, in the presence of D-APV, HFS triggered stabile potentiation accompanied by decreased

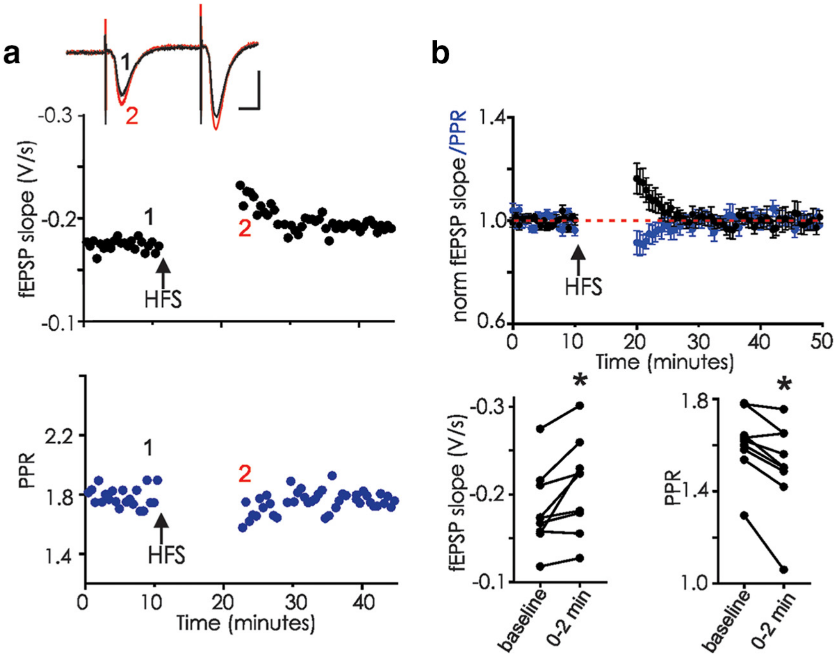

Figure 1. Labile LTP can be triggered by HFS of CA3-CA1 synapses in the presence of 50 μM D-APV. ***a***, fEPSPs in hippocampal area CA1 were evoked every 10 s. HFS (arrow; 100 Hz, 1 s repeated twice) elicited labile potentiation observable even after a 10 min pause without stimulation. Example from one hippocampal slice: Top (black), fEPSP; Bottom (blue), PPR. Inset, Average of 5 fEPSPs before (1, black) and just after resumption of stimulation after tetanus (2, red). Calibration: 10 ms, 0.5 mV. ***b***, Top, Average of 9 similar experiments showing mean ± SEM. Bottom, Raw data showing fEPSP slopes and PPR from each experiment ($n = 9$); fEPSPs, $p = 0.014$ for the first 2 min after resuming stimulation (paired *t* test). PPR, $p = 0.0041$ at 2 min after tetanus (paired *t* test). $^{*}p < 0.05$. In this and all experiments, 50 μM D-APV was present for at least 10 min before HFS.

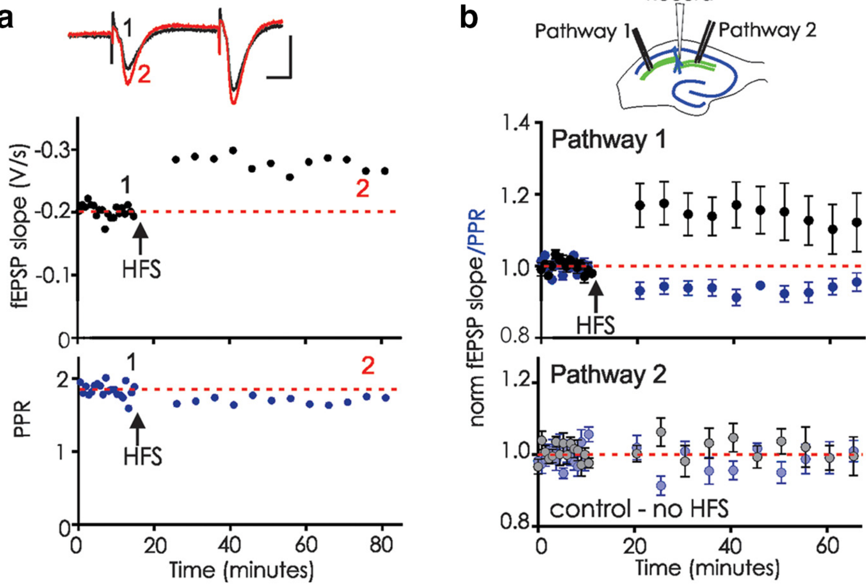

Figure 2. Labile LTP is synapse-specific and is maintained if post-HFS stimuli are delivered at low frequency. ***a***, Example experiment in which the baseline fEPSPs were evoked every 45 s. Post-HFS after a 10 min pause, stimulation was resumed at once every 5 min. Top, fEPSP slope. Bottom, PPR. Inset, Five averaged fEPSPs before (1, black) and at the end of the recording (2, red). Calibration: 10 ms, 0.5 mV. ***b***, Top, Diagram of recording arrangement with a stimulating electrode on either side of the extracellular recording site. Bottom, Experiments using two stimulation pathways. Pathway 1, fEPSP slope/PPR from the pathway that received HFS (arrow); Pathway 2, fEPSP slope/PPR from the control pathway in the same slice that did not receive HFS ($n = 9$, fEPSP in HFS vs control pathway, $p = 0.021$; PPR in HFS vs control pathway, $p = 0.0394$, unpaired *t* tests). Experiments that had potentiation of >10% in the control pathway (perhaps when the two pathways were not truly independent) were excluded from the dataset to emphasize that labile LTP is still observed in the HFS pathway even without potentiation of the control pathway.

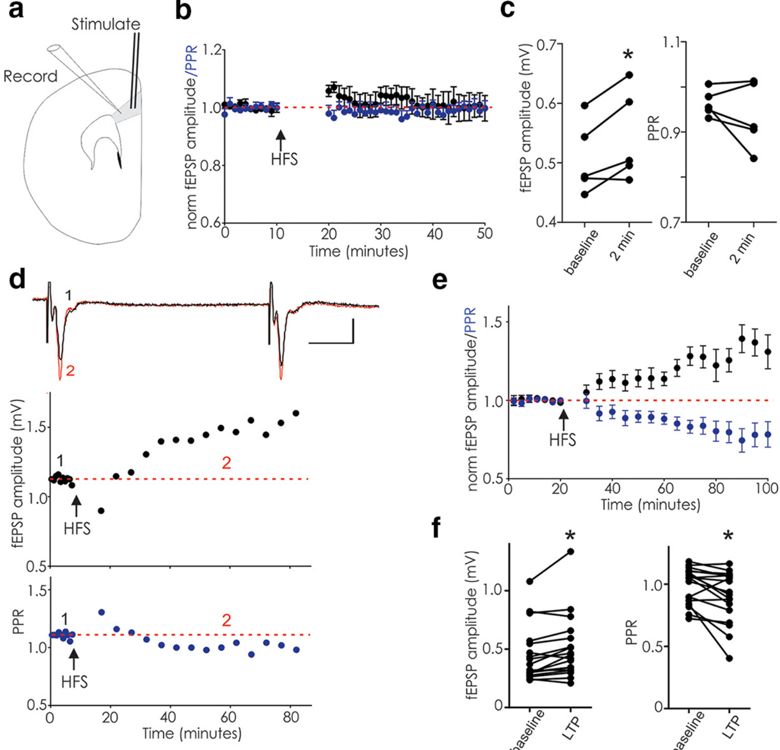

Figure 3. In PFC, HFS in D-APV elicits potentiation similar to hippocampal labile LTP. fEPSPs evoked in layer 2/3 and recorded in layer 5 of rat mPFC. ***a***, Diagram of electrode placements. ***b***, Average of 5 experiments illustrating transient potentiation of PFC fEPSPs after HFS (arrow) followed by a 10 min pause in stimulation; stimulation was at 0.1 Hz throughout. ***c***, Raw data for these experiments (fEPSPs, baseline vs first 2 min after HFS, $p = 0.031$; PPR, baseline vs first 2 min after HFS, $p = 0.313$). ***d***, Example experiment showing that HFS triggers potentiation when stimulation is resumed at once per 5 min following a 10 min pause. Inset, Average of 5 fEPSPs before (1, black) and during potentiation (2, red). Calibration: 10 ms, 0.5 mV. ***e***, Average of 18 similar experiments. ***f***, Raw data for each experiment in ***e*** (fEPSP amplitude, $p = 0.007$; PPR, $p = 0.009$; paired *t* tests). $^{*}p < 0.05$.

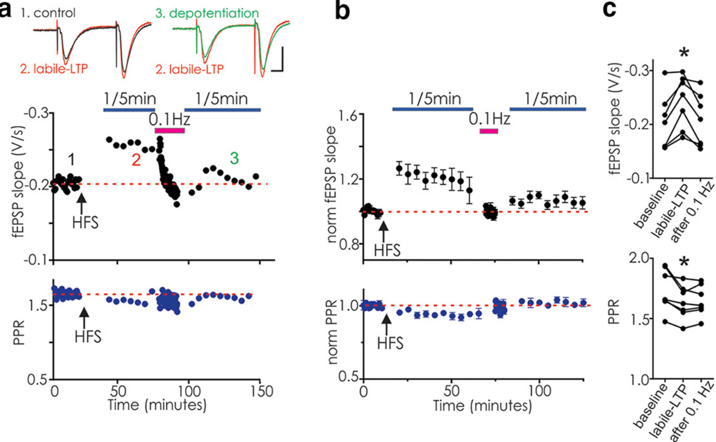

Figure 4. Synapses with labile LTP are depotentiated by stimulation at a higher frequency. CA3-CA1 synapses were stimulated once per 45 s before delivering HFS (arrow). After a 20 min pause, stimuli were resumed at 1 per 5 min for 50 min (blue bar); next, stimulus frequency was increased to 0.1 Hz for 10 min (magenta bar), and then after a 10 min pause, stimulus frequency was returned to once every 5 min (blue bar). ***a***, Example experiment demonstrating that labile LTP triggered by HFS returns to near baseline values after stimulation at 0.1 Hz. Top, Black symbols represent fEPSP. Bottom, Blue symbols represent PPR. Inset left, Average of 3 fEPSPs before (1, black) and during labile LTP (2, red). Inset right, Average of 3 fEPSPs during labile LTP and after 0.1 Hz stimulation (3, green, "depotentiation"). ***b***, Average of 7 similar experiments. Top, Black symbols represent fEPSP slope. Bottom, Blue symbols represent PPR. ***c***, Raw data showing fEPSP slopes ($F_{(1,8)} = 8.0$, $p = 0.014$; baseline vs labile LTP: $p = 0.0104$; baseline vs after 0.1 Hz: $p = 0.95$) and PPR from each experiment ($F_{(1,8)} = 9.41$, $p = 0.0105$; baseline vs labile LTP $p = 0.025$; baseline vs after 0.1 Hz, $p = 0.039$; repeated-measures one-way ANOVA followed by Dunnett's multiple-comparisons test). $^{*}p < 0.05$.

PPR. These data suggest that a form of potentiation similar to labile LTP also exists at mPFC synapses.

A remarkable feature of the persistent potentiation in hippocampus is that, once induced and monitored using very low-frequency stimulation, increasing the stimulus frequency returned the EPSPs and PPRs toward control levels (Fig. 4). One hypothesis to explain the origin of labile LTP is that the tetanus causes nascent synapses to form and become functional; these newly functional synapses might be more susceptible to presynaptic depression caused by vesicle depletion, and thus might seem to disappear upon resumption of higher stimulus frequencies (Xiao et al., 2004). To test this idea, we established labile LTP and then increased the stimulus frequency to produce depotentiation; we then halted stimulation for 10 min. Upon resuming stimulation at very low frequency, however, we did not recover potentiation (Fig. 4*a*–*c*). Our results suggest that nascent weak synapses or transient vesicle depletion cannot account for the depotentiation of synapses expressing labile LTP, but indicate instead that, once presynaptic action potential frequency reaches a certain threshold, the synapses are truly depotentiated.

The decreased PPR accompanying labile LTP indicates persistently increased presynaptic release (Felmy and von Gersdorff, 2006). Similarly, facilitation and PTP are also triggered by presynaptic trains of action potentials, are accompanied by a decreased PPR, and require elevated Ca^{2+} in the presynaptic terminal (Fioravante and Regehr, 2011). To test whether Ca^{2+} during the train is necessary for labile LTP, we next chelated Ca^{2+} using the membrane-permeant compound, EGTA-AM. As reported previously for hippocampal synapses (Ouanounou et al., 1996; Salin et al., 1996b; Nanou et al., 2016), bath application of EGTA-AM (200 μM) only modestly depressed the initial fEPSP but strongly attenuated paired pulse facilitation (Fig. 5*a*), presumably by decreasing the presynaptic Ca^{2+} required for facilitation (Atluri and Regehr, 1996). In the presence of EGTA-AM, labile LTP was blocked; and in some experiments, synaptic depression was observed after HFS (Fig. 5*b*, top). To ensure that EGTA-AM had reached a functional steady state, we instead preincubated slices for 1 h before the start of recording; in the continued presence of EGTA-AM, labile LTP was not induced (Fig. 5*b*, bottom). EGTA-AM will chelate Ca^{2+} in both presynaptic and postsynaptic neu-

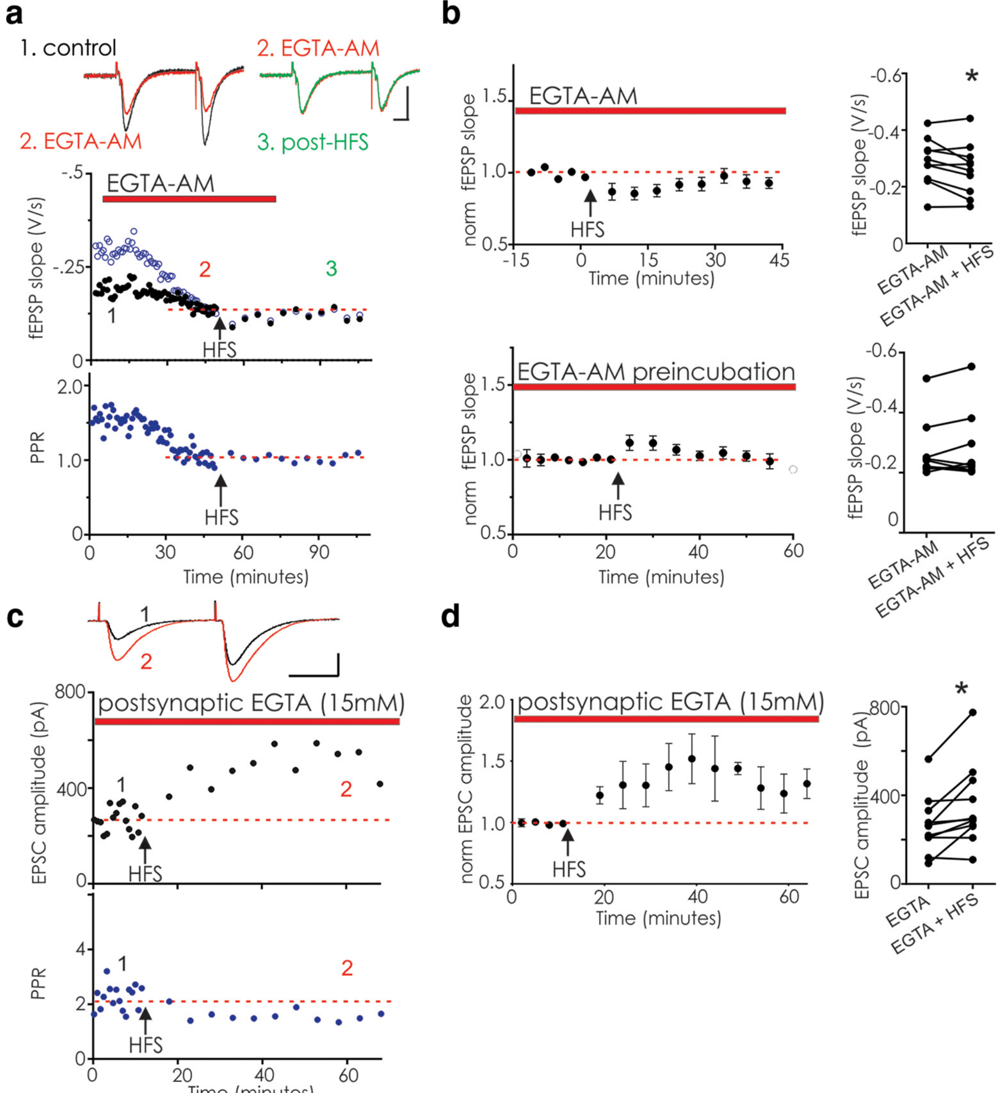

Figure 5. Labile LTP is blocked by bath-applied EGTA-AM but not by postsynaptically delivered EGTA. ***a***, Example experiment showing acute bath application of 200 μM EGTA-AM to a slice. Top, fEPSP slope. Black symbols represent fEPSP1. Open blue symbols represent fEPSP2. Bottom, PPR for the same experiment. EGTA-AM decreased the second fEPSP with little effect on fEPSP1 (1, control; 2, EGTA-AM). Subsequent HFS did not elicit labile LTP nor a decrease in the PPR (3, post-HFS). Inset, Average of 5 fEPSPs before EGTA-AM application (black), as well as before (red) and after HFS (green). Calibration: 10 ms, 0.5 mV. ***b***, Top left, Average of 10 similar experiments, illustrating the last 15 min of baseline recording in EGTA-AM; EGTA-AM was applied for at least 30 min before HFS. Right, Raw data from each experiment. On average, HFS did not elicit labile LTP but instead elicited a significant depression (all points after HFS over the next 45 min; $p = 0.043$, paired *t* test). Bottom left, Averaged data for 9 experiments for which slices were incubated for 60 min in EGTA-AM before being recorded from in the continued presence of EGTA-AM. Right, Raw data from each experiment. HFS did not induce labile LTP ($p = 0.44$, paired *t* test). Potentiation following HFS was significantly reduced in both acutely applied EGTA-AM and preincubation experiments compared with labile LTP in the 9 slices in Figure 2 that had HFS (acute, $p = 0.0003$; preincubated, $p = 0.039$; unpaired *t* test). ***c***, Example whole-cell voltage-clamp recording from a CA1 pyramidal cell with EGTA (15 mM) included in the intracellular pipette solution. HFS was delivered at least 20 min after break-in to ensure diffusion of EGTA. Inset, Average of 5 EPSCs comparing baseline (1, black) with labile LTP (2, red). Calibration: 20 ms, 200 pA. Top, Black symbols represent EPSC. Bottom, Blue symbols represent PPR. ***d***, Left, Average of 9 similar experiments with 15 mM EGTA in the pipette (EPSC after HFS vs baseline, $p = 0.010$, paired *t* test). Right, Raw data from each experiment. *$p < 0.05$.

rons in these experiments. We next selectively chelated Ca^{2+} in the postsynaptic neuron by including 15 mM EGTA in the recording pipette during whole-cell recordings from CA1 pyramidal neurons before HFS. Labile LTP was robustly induced in these experiments (Fig. 5*c*,*d*), demonstrating that postsynaptic Ca^{2+} is not required for labile LTP induction, and instead suggesting that the block of labile LTP by EGTA-AM may result from chelation of presynaptic Ca^{2+}.

While chelation of Ca^{2+} by EGTA-AM may have multiple effects, potentially inhibiting the release of most neurotransmitters and modulators, as well as other signaling molecules, the decreased PPR and block of labile LTP by EGTA-AM are consistent with the idea that a Ca^{2+}-dependent process in the presynaptic terminal underlies labile LTP. We next explored the involvement of several candidate presynaptic proteins implicated in short-term synaptic plasticity.

RIM1α is a central component of the release machinery in nerve terminals, interacting with multiple proteins, including the synaptic vesicle proteins Rab3 and synaptotagmins as well as munc-13, ELKS, and liprins (Schoch et al., 2002). RIM1α knock-out mice lack LTP at several synapses in which LTP is known to require a rise in presynaptic Ca^{2+} and is maintained by presynaptic increases in glutamate release (Salin et al., 1996a; Castillo et al., 2002; Fourcaudot et al., 2008). Because of these similarities with labile LTP, we next examined slices from RIM1α knock-out mice. Robust labile LTP was triggered in synapses from RIM1α

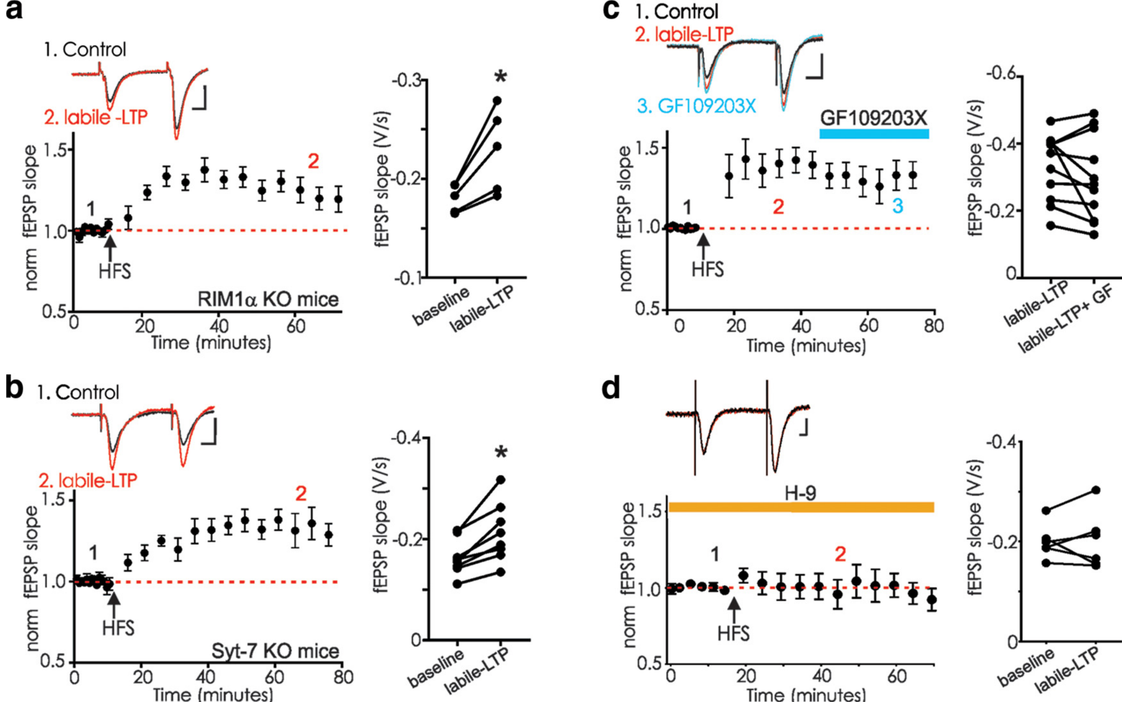

Figure 6. Labile LTP is intact in RIM1α or syt-7 knock-out mice but blocked by a protein kinase inhibitor. ***a***, Hippocampal slices from RIM1α KO mice express labile LTP after tetanus ($n = 5$, $p = 0.019$, paired *t* test). Inset, fEPSPs before (1, black) and after HFS (2, red). ***b***, Hippocampal slices from syt-7 KO mice express labile LTP after HFS ($n = 7$, $p = 0.002$, paired *t* test). Inset, fEPSPs before (1, black) and after HFS (2, red). ***c***, The PKC inhibitor GF109203X (10 μM) was added to the bathing medium once labile LTP was established in rat hippocampal slices ($n = 11$, labile LTP before and after 30 min GF, $p = 0.20$, paired *t* test). Inset, fEPSPs before (1, black), during labile LTP (2, red), and after 10 min GF109203X (3, blue). ***d***, The broad-spectrum kinase inhibitor H-9 (200 μM) was added to the bathing medium at least 20 min before induction of labile LTP in rat hippocampal slices ($n = 6$, $p = 0.91$, paired *t* test). Inset, fEPSPs before HFS and (1, black) and after HFS in H-9 (2, red). Calibration: 0.5 mV, 10 ms. ***a–d***, Right, Raw data for each experiment. $^*p < 0.05$.

knock-out mice (Fig. 6*a*), suggesting that this form of potentiation is mechanistically different from other presynaptically maintained forms of LTP.

Syt-7 is a Ca^{2+}-binding protein found at many central synapses that contributes to presynaptic asynchronous transmitter release as well as postsynaptic AMPAR trafficking (Sugita et al., 2001; Li et al., 2017; Wu et al., 2017). It was recently shown that several different synapses in the syt 7 knock-out mouse have markedly attenuated facilitation, among these the CA3-CA1 synapse that supports labile LTP (Jackman et al., 2016; Turecek et al., 2017), and we therefore next measured labile LTP in the syt-7 knock-out mouse. As reported, paired pulse facilitation was nearly absent at the CA3-CA1 synapse in slices from these animals; however, labile LTP appeared unaffected (Fig. 6*b*). Despite their shared roles in plasticity involving release probability and presynaptic Ca^{2+}, neither RIM1α nor syt-7 is necessary for labile LTP.

The long period following the HFS before resuming stimulation in our experiments makes it unlikely that accumulated Ca^{2+} remains in the terminal, but persistent activation of a Ca^{2+}-dependent enzyme, such as PKC, could account for the maintained potentiation. PTP can be blocked by the PKC inhibitor, GF109203X, and this compound blocks PTP at CA3-CA1 synapses (Brager et al., 2003; Korogod et al., 2007; Wierda et al., 2007; Wang et al., 2016). However, once labile LTP was established, 10 μM GF109203X did not significantly reverse the potentiation (Fig. 6*c*). To further test the role of protein kinases in the induction of labile LTP, we next bath-applied the broad-spectrum kinase inhibitor, H-9. At 200 μM, this kinase is expected to block PKA, PKG, PKC, and CaMKII. H-9 effectively blocked the induction of labile LTP (Fig. 6*d*).

Discussion

We have characterized a novel form of synaptic potentiation at hippocampal synapses that differs from other forms of potentiation. Like other examples of LTP, labile LTP is maintained after it is triggered without further synaptic activity, even after a long silent period. However, unlike LTP, if the synapses are active after its induction, even at 0.1 Hz, depotentiation occurs within a few minutes (Volianskis and Jensen, 2003), but labile LTP will remain stable with stimulation at 0.0033 Hz. Labile LTP is synapse-specific, as it is not observed at neighboring unstimulated synapses.

Mechanisms underlying labile LTP

Mechanistically, labile LTP does not require NMDAR activation, and thus is distinct from short-term potentiation (Kauer et al., 1988; Volianskis et al., 2013a) or NMDAR-dependent LTP (Collingridge et al., 1983). Despite indications that labile LTP may involve GluN2B (Volianskis et al., 2013b), receptors including this subunit are largely blocked by 50 μM D-APV (Buller et al., 1994); we thus conclude that NMDARs are not required for the labile LTP we characterized. If NMDARs are present on presynaptic terminals at a given synapse (Berretta and Jones, 1996; McGuinness et al., 2010), they could theoretically boost labile LTP magnitude by increasing presynaptic Ca^{2+}, and thus increase the magnitude of labile LTP (sometimes called short-term potentiation) observed in earlier studies (Volianskis and Jensen, 2003; Volianskis et al., 2013b). This would not have been seen in our study as D-APV was present throughout all experiments. Furthermore, labile LTP is sustained in the presence of D-APV when synapses are stimulated infrequently (once per 5 min, 0.00334 Hz); in earlier experiments, more frequent stimulation after in-

duction may have contributed to synapse depotentiation (Volianskis and Jensen, 2003).

The correlated reduction in the PPR suggests a presynaptic locus for labile LTP maintenance. Bath-applied EGTA-AM, but not postsynaptically delivered EGTA, prevented labile LTP induction, consistent with a requirement for elevated presynaptic Ca^{2+}. Labile LTP was still induced under conditions in which PTP and facilitation are attenuated or absent, demonstrating that it is mechanistically distinct from these presynaptic processes and at least some of their underlying proteins. RIM1α and syt-7, presynaptic proteins implicated in the control of neurotransmitter release, are not required for labile LTP, and PKC is not essential for labile LTP maintenance. Our results using a broad-spectrum kinase inhibitor, H-9, suggest that kinase activation is necessary for labile LTP. We speculate that a Ca^{2+}-sensitive enzyme is the most likely candidate to mediate labile LTP induction. Multiple Ca^{2+}-dependent protein kinases, including CaMKII, PKC, and myosin light-chain kinase, can increase release probability (de Jong and Verhage, 2009). Mice lacking synapsins exhibit attenuated short-term synaptic plasticity notable after HFS in hippocampal slices (Rosahl et al., 1993, 1995), hinting at a potential role for these vesicle-associated phosphoproteins in labile LTP. Depotentiation after labile LTP also appears to be a Ca^{2+}-dependent process; in hippocampal synapses, 0.133 Hz stimulation depotentiated labile LTP, even with glutamate receptors blocked by kynurenic acid and LY341495; however, the same stimulation in zero-Ca^{2+} solution did not permit depotentiation (Volianskis and Jensen, 2003). Together with the block of labile LTP by EGTA-AM reported here, this observation indicates a requirement for Ca^{2+} both to initiate labile LTP and for depotentiation. Further work will be needed to identify the proteins that control both processes.

Role of labile LTP in hippocampal and prefrontal circuitry

Labile LTP was originally described using field potential recordings from rat hippocampal slices, and we now demonstrate that labile LTP can also be observed in mouse hippocampus and in whole-cell recordings, and a labile potentiation with similar features can be elicited in prefrontal cortical synapses. Its intriguing properties may allow labile LTP to contribute to a variety of network processes. Labile LTP would be expected to tag synapses that have recently been highly active, increasing their probability of release for a defined period. At hippocampal and cortical synapses with relatively low release probability (Rosenmund et al., 1993; Dobrunz and Stevens, 1997), such a transient tag may permit specific inputs to increase their typical signal-to-noise ratio. This in turn is expected to increase the likelihood of coincident glutamate release and postsynaptic depolarization, promoting induction of NMDAR-dependent LTP. In the hippocampus, labile LTP could contribute to retention of information at specific synapses for short periods. This might be useful for hippocampal function during episodic learning, maintaining temporal order information, or perhaps could contribute to pattern completion or trace conditioning (Cohen and Eichenbaum, 1991; Shors, 2004; Kesner and Hopkins, 2006; Cowan, 2008). CA3 pyramidal cells fire *in vivo* in bursts at ~200 Hz, often with ≥6 action potentials per bursts, repeated every ~1–2 s (Kowalski et al., 2016). The 100 Hz HFS protocol used here to induce labile LTP is of longer duration than likely occurs *in vivo*, but the frequency is in the physiological range. Labile LTP can also be elicited with 30 Hz afferent stimulation or a theta burst protocol, demonstrating that it can be triggered by various patterns of presynaptic activity at relatively high frequency (Volianskis and Jensen, 2003).

The PFC is generally thought to have a key role in working memory (Fuster, 2009; D'Esposito and Postle, 2015). Recently, there has been considerable discussion of the possibility that working memory may not result solely from persistent neuronal firing, and memory may remain even after pauses in the persistent activity (LaRocque et al., 2013; Stokes, 2015). We observed potentiation with some features reminiscent of hippocampal labile LTP at layer 2–3 to layer 5 PFC synapses, where it could contribute to working memory, retaining information at recently active synapses without the expense of persistently active firing in cells of the relevant network.

Although not yet tested explicitly, labile LTP may enable high-frequency activation of a single axon to elevate the release probability for a period of time dependent on subsequent presynaptic activity of that axon. This nonassociative arrangement is different from NMDAR-dependent forms of plasticity in its independence of the postsynaptic neuron, instead depending in theory only on presynaptic afferent activity. Labile LTP cannot on its own account for acquisition of novel associations, but instead would simply retain the information of recent local network activity and could contribute to nonassociative retention of information over short time periods.

In many electrophysiological experiments, labile LTP can be masked by other short- and long-term plasticity phenomena, and so may have easily been overlooked. The observation of labile LTP at synapses in both hippocampal and cortical circuits raises the question of how widespread this phenomenon is. For example, do CA3 or layer 2/3 afferents synapsing on GABAergic interneurons also exhibit labile LTP upon HFS? Do GABAergic nerve terminals exhibit labile LTP? Our results suggest a requirement for presynaptic Ca^{2+} and activation of kinases to trigger labile LTP, but beyond that our experiments have only ruled out several likely participants. Understanding the mechanisms that underlie labile LTP should clarify its behavioral functions and how it may be used by neurons embedded in distinct circuits.

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