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Partial inhibition of PP1 alters bidirectional synaptic plasticity in the hippocampus

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Abstract

Synaptic plasticity is an important cellular mechanism that underlies memory formation. In brain areas involved in memory such as the hippocampus, long-term synaptic plasticity is bidirectional. Major forms of bidirectional plasticity encompass long-term potentiation (LTP), LTP reversal (depotentiation) and long-term depression (LTD). Protein kinases and protein phosphatases are important players in the induction of both LTP and LTD, and the serine/threonine protein phosphatase-1 (PP1), in particular, has emerged as a key phosphatase in these processes. The goal of the present study was to assess the contribution of PP1 to bidirectional plasticity and examine the impact of a partial inhibition of PP1 on LTP, LTD and depotentiation in the mouse hippocampus. For this, we used transgenic mice expressing an active PP1 inhibitor (I-1*) inducibly in forebrain neurons. We show that partial inhibition of PP1 by I-1* expression alters the properties of bidirectional plasticity by inducing a shift of synaptic responses towards potentiation. At low-frequency stimulation, PP1 inhibition decreases LTD in a frequency-dependent fashion. It favours potentiation over depression at intermediate frequencies and increases LTP at high frequency. Consistently, it also impairs depotentiation. These results indicate that the requirement of bidirectional plasticity for PP1 is frequency-dependent and that a broad range of plasticity is negatively constrained by PP1, a feature that may correlate with the property of PP1 to constrain learning efficacy and promote forgetting.

Introduction

The hippocampus is an essential brain structure for the formation of explicit memory. It displays two major forms of long-term synaptic plasticity, long-term potentiation (LTP) and long-term depression (LTD). In the CA1 region, both LTP and LTD can be evoked at the same synapses by stimulating Schaffer collaterals with different frequencies. Whereas high-frequency stimulation induces LTP, low-frequency stimulation induces LTD. Although the precise molecular mechanisms underlying these forms of plasticity are not fully understood, it is known that Ca^{2+} influx into postsynaptic cells is essential (Lisman, 1989). Ca^{2+} influx is shaped by the strength of stimulation and, in turn, its level determines which downstream components of Ca^{2+}-dependent postsynaptic signalling cascades are activated. Thus, whereas high frequency leads to strong Ca^{2+} influx and preferentially activates protein kinases and LTP (Malenka *et al*., 1989), low frequency induces weak Ca^{2+} influx and activates protein phosphatases that favour LTD (Isaac, 2001; Morishita *et al*., 2001; Winder & Sweat, 2001). Among the protein phosphatases present in neuronal cells, the highly Ca^{2+}-sensitive protein phosphatase calcineurin (PP2B) is known to be one of the most critical for LTD. Protein phosphatase-1 (PP1), another serine/threonine phosphatase, has also emerged as a prominent regulator of synaptic plasticity. PP1 is a multifunctional enzyme that controls the phosphorylation and activity of a variety of effectors governing synaptic strength (Greengard *et al*., 1999). It has been shown to gate LTP and needs to be inhibited for LTP induction and expression (Blitzer *et al*., 1998; Woo *et al*., 2002). Conversely, PP1 sustains LTD and remains active during LTD expression by binding to cognate targeting proteins (Morishita *et al*., 2001, 2005). PP1 is also implicated in cognitive functions and has been shown to limit learning and favour forgetting. Its partial inhibition in the brain (about 68% in hippocampus and 45% in cortex) by expression of a selective PP1 inhibitor (I-1*) was found to improve learning and memory in young adult and aged mice, an effect that correlates with increased phosphorylation of several targets including the Ca^{2+}/calmodulin-dependent protein kinase CaMKII, the alpha-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate (AMPA) receptor subunit GluR1 and the transcription factor CREB (Genoux *et al*., 2002).

The aim of the present study was to examine whether the improved cognitive performance observed in the I-1* mutant mice correlates with changes in basal synaptic transmission and plasticity. We were particularly interested in bidirectional plasticity, a property of synaptic circuits thought to be essential for information processing and memory storage.

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Materials and methods

Generation of transgenic mice

Mice expressing a constitutively active form of inhibitor-1 (I-1*) in a doxycycline (dox)-dependent fashion in forebrain neurons (Genoux *et al*., 2002) were obtained by breeding transgenic mice carrying the reverse tetracycline-controlled transactivator (rtTA) gene driven by the CaMKIIα promoter with mice carrying a tetO promoter linked to I-1* cDNA. Double transgenic animals (mutant) express I-1* when fed dox-medicated food. Mutant and control littermates received *ad libitum* either regular food or dox-medicated food (Mutual Pharmaceutical Co., Philadelphia, PA, USA; 6 mg/g of food) prepared daily and administered for at least 6 days prior to experimentation. Single transgenic mice treated or not treated with dox were used as controls. All experimental procedures involving animals and animal care were conducted according to INSERM committee guidelines and European Communities Council Directive 86/609/EEC (24 November 1986).

Slice preparation

Mice (6–9 months old) were anaesthetized with halothane and decapitated. The hippocampus was quickly removed and placed in cold medium containing 124 mM NaCl, 3.5 mM KCl, 1.5 mM $MgSO_4$, 2.5 mM $CaCl_2$, 26.2 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 11 mM glucose. Slices (400 μm thick) were prepared and placed in a holding chamber (at 27 °C) at least 1 h before recording. Each slice was individually transferred to a submersion-type recording chamber and submerged with medium continuously superfused and equilibrated with 95% O_2, 5% CO_2. Okadaic acid and KT5720 (Biomol, Le Perray-en-Yveline, France) were diluted in medium from stock solutions prepared in DMSO.

Electrophysiological recordings

Extracellular recordings were performed at room temperature in apical dendritic layers of CA1 area using glass micropipettes filled with 2 M NaCl with a resistance of 2–6 MΩ. Field excitatory postsynaptic potentials (fEPSPs) were evoked by electrical stimulation (bipolar electrode) of CA1 afferent Schaffer collaterals and commissural fibres in the stratum radiatum. Test stimuli (100 μs duration) were adjusted to obtain an fEPSP slope of 30% of maximum response and applied every 10 s (in LTD experiments) or 30 s (in LTP experiments). The magnitude of fEPSPs was determined by measuring the slope of fEPSPs and was expressed across time. Experiments were performed by experimenters blind to genotype whenever possible.

Input/output

Curves were constructed to assess the responsiveness of AMPA/kainate glutamate receptor subtype-dependent responses to electrical stimulation in slices. The amplitude of three averaged presynaptic fibre volleys (PFVs) and the slope of fEPSPs were plotted as a function of stimulation intensity (30–300 μA). A similar experiment was conducted in low magnesium (0.1 mM) in the presence of non-*N*-methyl-D-aspartate (NMDA) receptor antagonist CNQX (10 μM). fEPSPs recorded under these conditions are mediated by NMDA receptors.

Paired-pulse facilitation (PPF)

PPF of synaptic transmission induced by paired-pulse stimulation was monitored across a range of interstimulus intervals (ISIs) from 10 to 400 ms. PPF was quantified by normalizing the slope of the second response by the slope of the first one, and plotted against ISI.

Long-term depression

LTD was induced by applying low-frequency stimulation (LFS) to Schaffer collaterals (900 pulses, 1 Hz or 1200 pulses, 2 Hz) after baseline recording (at least 15 min or until stable) and was measured by single pulse testing for at least 45 min after LFS.

Long-term potentiation and depotentiation

LTP was induced by applying high-frequency stimulation (HFS) to Schaffer collaterals (one, two or four trains of 1 s at 100 Hz separated by 20 s or 5 min) after baseline recording (at least 15 min or until stable). Three successive fEPSPs were calculated and the resulting average slope was plotted online across time using Acquis 1 software (G Sadoc, UNIC-CNRS, France). Recording after single pulse testing was performed for at least 60 min following tetanus. Depotentiation was induced either 5 or 30 min after LTP induction (two trains of 1 s at 100 Hz with 20 s ISI) by applying LFS (900 pulses, 1 Hz or 1200 pulses, 2 Hz at test intensity) and recorded for at least 45 min after LFS.

Data analysis

For LTP, LTD and depotentiation, average fEPSPs slope was calculated for 10 min starting 35 min after stimulation and was expressed as percentage of baseline response (% baseline) ± SEM. Statistical significance was assessed using one-factor analysis of variance (ANOVA) or ANOVAs for repeated measures data.

Results

Basal synaptic transmission is not altered by partial inhibition of PP1

The effect of I-1* transgene expression on synaptic transmission was investigated by comparing the size of fEPSPs and PFVs in I-1* control and mutant hippocampal slices. fEPSP and PFV responses increased similarly as a function of intensity in both I-1* mutant ($n = 10$ slices from four mice) and control ($n = 7$ slices from three mice) slices (intensity effect: $P < 0.001$, intensity by genotype fEPSPs: $F_{1,15} = 1.01$, n.s.; PFVs: $F_{1,15} = 1.3$, n.s.). Furthermore, the regression line of the function relating fibre volley amplitude to fEPSP slope, an index of basal synaptic strength (Fig. 1A) and the magnitude of NMDA receptor-dependent responses were similar in control ($n = 7$) and mutant ($n = 6$) slices, regardless of stimulation intensity (Fig. 1B) ($F_{1,11} = 0.27$, n.s.). In addition, PPF, a short-lasting enhancement in synaptic strength essentially mediated by presynaptic calcium-dependent mechanisms (Kamiya & Zucker, 1994), was similar in control ($n = 9$ slices from four mice) and mutant ($n = 7$ slices from four mice) slices ($F_{1,14} = 0.66$, n.s.; Fig. 1C).

PP1 inhibition modulates plasticity in a frequency-dependent fashion

LTD

We examined plasticity in hippocampus area CA1 across a wide range of stimulation frequencies. We first looked at LTD using 1 or 2 Hz stimulation. Stimulation at 1 Hz (900 pulses, 15 min) significantly

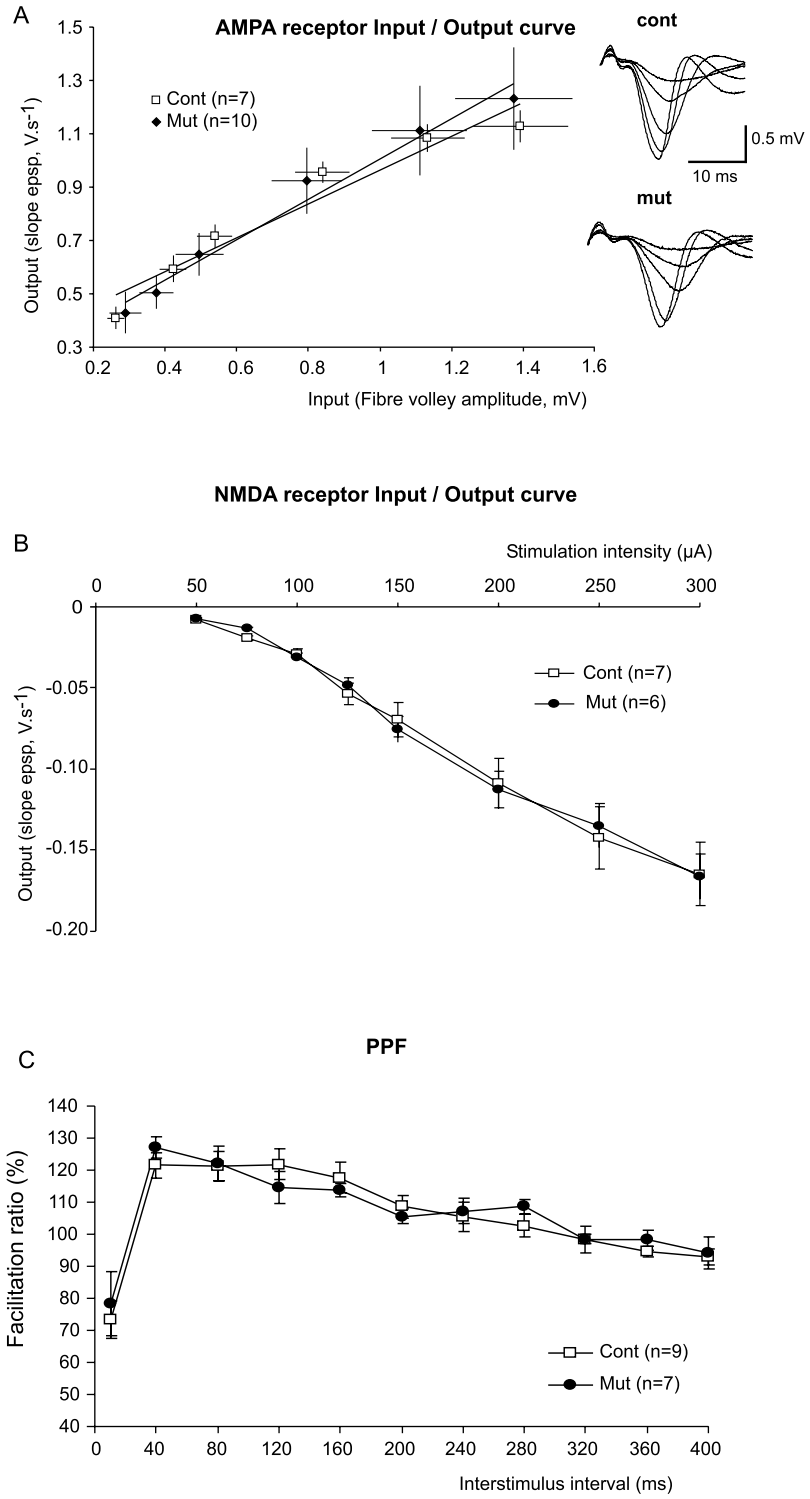

FIG. 1. Basal synaptic transmission is not altered by PP1 inhibition. (A) Mean fEPSP slopes of AMPA receptor-mediated synaptic responses against presynaptic fibre volley amplitude in hippocampal slices from control (Cont, open squares, fit with regression line $y = 0.64x + 0.33$, $r^2 = 0.935$) and mutant (Mut, filled diamonds, fit with regression line $y = 0.75 + 0.25$, $r^2 = 0.978$) mice. Inset shows individual traces for control and mutant slices. (B) Mean fEPSP slopes of NMDA receptor-mediated synaptic responses against current intensity recorded in low magnesium in the presence of the AMPA receptor antagonist CNQX (10 µM). (C) Mean paired-pulse facilitation (PPF) of fEPSP slope as a function of ISIs.

reduced fEPSPs in both I-1* mutant and control slices and induced similar LTD in both groups (mutant, 81.9 ± 3.0% of baseline, $n = 15$ slices from seven mice; control, 86.3 ± 4.2% of baseline, $n = 12$ slices from seven mice; $F_{1,25} = 0.90$, n.s.; Fig. 2, A and A1). However, when stimulation was raised to 2 Hz (1200 pulses, 10 min), a stronger LTD was observed in control slices (70.2 ± 2.9% of baseline, $n = 20$ slices from 15 mice; 2 Hz vs. 1 Hz, $F_{1,30} = 11.4$, $P < 0.01$) but not in mutant slices, in which fEPSPs remained similar

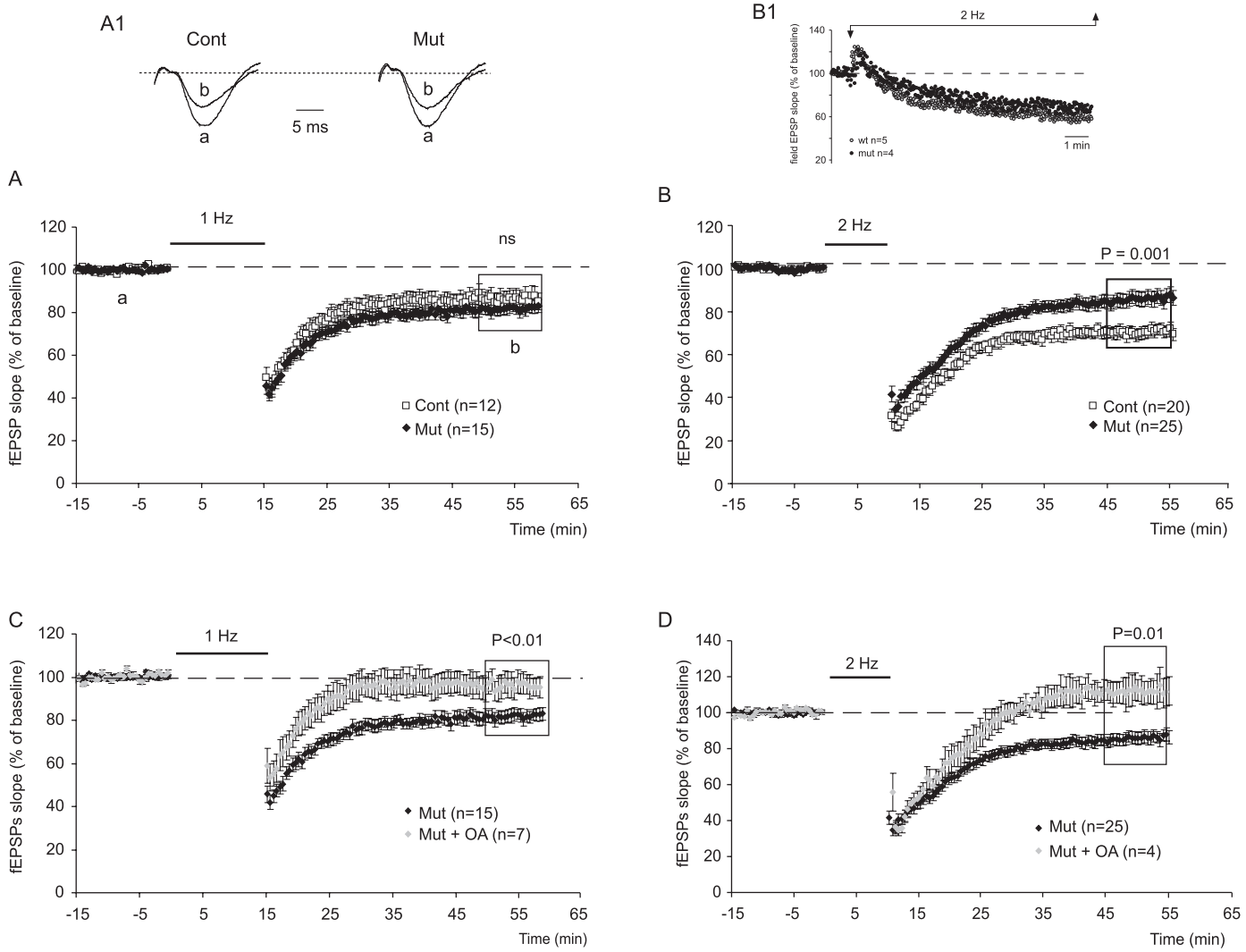

FIG. 2. PP1 inhibition modulates LTD in a frequency-dependent fashion. LTD induced by (A) 1 Hz for 15 min or (B) 2 Hz for 10 min in hippocampal slices from I-1* control (open squares) and mutant mice (filled diamonds). 1 Hz stimulation induces similar LTD in control and mutant slices but 2 Hz stimulation induces weaker depression in mutant slices. A1 shows individual fEPSPs traces (a) before and (b) after stimulation in control and mutant slices. B1 shows fEPSPs slope during 2 Hz stimulation as representative for similar stimulation efficacy in both groups. (C and D) Blockade of residual LTD in I-1* mutant slices following stimulation at 1 Hz (C) or 2 Hz (D) (filled diamonds) by pre-incubation of slices (for 2 h) in okadaic acid (1 μM) (shaded diamonds). Black frames indicate the time window of measurement of mean fEPSP slope (over 10 min starting 35 min after stimulation) with *P*-values. n.s., non-significant.

to that at 1 Hz (86.4 ± 3.5% of baseline, $n = 25$ slices from 14 mice; 2 Hz vs. 1 Hz, $F_{1,38} = 0.60$, n.s.; Fig. 2B). Thus, at 2 Hz, LTD was significantly lower in mutant slices than control slices ($F_{1,43} = 12$, $P = 0.001$). During stimulation (10 min for 2 Hz), a similar decrease in fEPSP slope was observed in both groups (Fig. 2, B1), indicating no gross alteration of presynaptic mechanisms by PP1 inhibition.

The different impact of 1 and 2 Hz stimulation in I-1* mutant and control slices suggests that different mechanisms may be recruited by these stimulation frequencies. Thus, although both forms of LTD may depend on PP1, 1 Hz stimulation may need only a small fraction of PP1 and may not be affected by partial inhibition of PP1 (68% in I-1* mutant hippocampus, Genoux *et al*., 2002). By contrast, 2 Hz may require full PP1 activity, and be limited in its expression by insufficient PP1 activity due to inhibition by I-1*. To test this possibility, we repeated the 1 Hz experiment in mutant slices in the presence of the PP1/PP2A inhibitor okadaic acid (1 μM) to block the residual PP1 activity. Under these conditions, LTD was fully abolished (96.0 ± 5.2% of baseline, $n = 7$ slices from four mice; $F_{1,20} = 7.3$, $P = 0.01$; Fig. 2C). Okadaic acid also prevented 2 Hz LTD in control slices (111.8 ± 7.5% of baseline, $n = 4$ slices from five mice; $F_{1,27} = 8.9$, $P < 0.01$; Fig. 2D), indicating that both 1- and 2-Hz LTD are PP1-dependent but have different requirements for PP1.

LTD/LTP threshold

We next examined whether the genetic inhibition of PP1 also alters plasticity at frequencies near the LTD/LTP modification threshold by stimulating Schaffer collaterals at intermediate frequencies. Stimulation at 5 Hz induced a depression in both control and mutant slices that was weaker in mutant slices (control, 75.02 ± 2.8% of baseline, $n = 12$ slices from five mice; mutant, 94.3 ± 5.7% of baseline, $n = 7$ slices from three mice; $F_{1,17} = 13.68$, $P < 0.001$; Fig. 3A). Stimulation at 10 Hz (one train of 900 pulses) elicited a depression in control slices, but it induced a slight potentiation in mutant slices (mutant, 106.3 ± 4.4% of baseline, $n = 7$ slices from four mice; control, 78.3 ± 3.3% of baseline, $n = 16$ slices from eight mice;

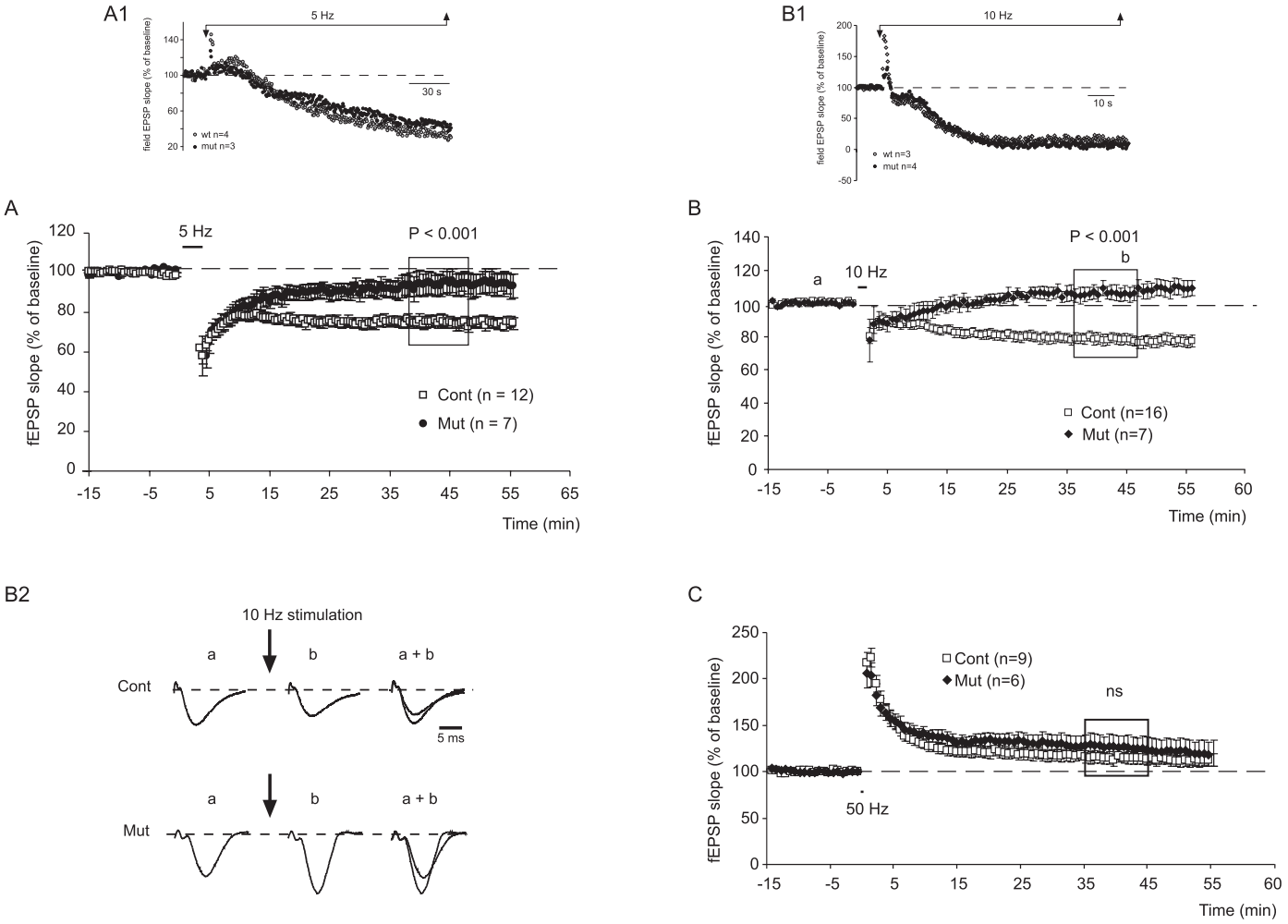

FIG. 3. PP1 inhibition shifts plasticity towards potentiation at the modification threshold. Plasticity induced by (A) 5 Hz for 3 min, (B) 10 Hz for 1.5 min, or (C) 50 Hz for 2 s in I-1* control (open squares) and mutant hippocampal slices (filled diamonds). 5 Hz stimulation induces a depression in control slices but no change in plasticity in mutant slices. 10 Hz stimulation induces a depression in control slices but a potentiation in mutant slices while 50 Hz stimulation induces similar potentiation in control and mutant slices. fEPSP slope during stimulation at 5 Hz (A1) and 10 Hz (B1) indicates similar stimulation efficacy in both groups. B2 shows individual fEPSP traces before (a) and after (b) stimulation in control and mutant slices. Black frames indicate the time window of measurement of mean fEPSP slope (over 10 min starting 35 min after stimulation) with *P*-values. n.s., non-significant.

$F_{1,21} = 27.07$, $P < 0.001$; Fig. 3, B and B2). At a higher frequency (50 Hz), fEPSPs were potentiated to a similar extent in control and mutant slices (control, 114.7 ± 6.5% of baseline, $n = 9$ slices from seven mice; mutant, 126.0 ± 12.6% of baseline, $n = 6$ slices from three mice; $F_{1,18} = 2.78$, n.s.; Fig. 3C). No difference in fEPSP slope during stimulation (whether 5 or 10 Hz) was observed between control and mutant slices (Fig. 3, A1 and B1).

LTP

At 100 Hz, a slightly more robust LTP was induced in mutant slices compared with controls using one or two trains (5-min ISI) of 100 pulses (one train: mutant, 136.9 ± 5.6% of baseline, $n = 6$ slices from five mice; control, 121.1 ± 3.8% of baseline, $n = 12$ slices from 12 mice; $F_{1,16} = 7.045$, $P < 0.05$; Fig. 4A; two trains: mutant, 146.5 ± 7.3% of baseline, $n = 7$ slices from six mice; control, 130.2 ± 4.9% of baseline, $n = 12$ slices from 11 mice; $F_{1,17} = 4.11$, $P = 0.05$; Fig. 4B). This increase in potentiation was not seen with four trains of 1 s stimulation (5-min ISIs), which elicited a similar, probably saturated, LTP in mutant and control slices (mutant, 145.6 ± 5.6% of baseline, $n = 11$ slices from seven mice, control, 147.9 ± 7.1% of baseline, $n = 11$ slices from ten mice; $F_{1,20} = 0.078$, n.s.; Fig. 4C). Notably, there was a significant frequency-dependent increase in LTP in control slices (frequency effect: $F_{2,32} = 6.25$, $P < 0.01$) but not in mutant slices (frequency effect: $F_{2,20} = 0.658$, n.s.). Rather, in mutant slices, maximum potentiation of fEPSP slope was already induced after one train of HFS.

Overall, when the results were plotted on a frequency–response curve, a clear response shift towards potentiation was revealed across a wide range of stimulation frequencies (2–30 Hz) in I-1* mutant slices compared with control slices (Fig. 5).

Depotentiation is altered by PP1 inhibition

To evaluate further the effect of PP1 inhibition on bidirectional plasticity, we examined whether depotentiation, a form of LTP reversal, is altered in I-1* mutant slices. Previous studies have shown that depotentiation is NMDA receptor-dependent and shares common properties with LTD (Bear & Abraham, 1996; Huang & Hsu, 2001). Depotentiation was elicited in area CA1 by first inducing LTP followed 5 or 30 min later by LFS at 1 or 2 Hz. LTP was induced with

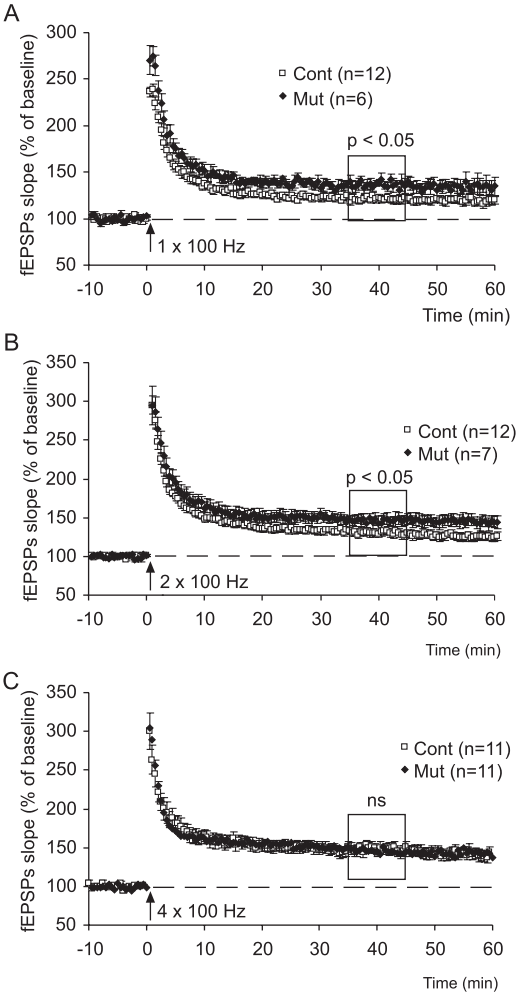

FIG. 4. LTP is enhanced by PP1 inhibition. LTP induced by (A) one train (1 s) at 100 Hz, (B) two trains (1 s each) at 100 Hz, 5 min ISI, or (C) four trains (1 s each) at 100 Hz, 5 min ISIs in I-1* control (open squares) and mutant slices (filled diamonds). One- and two-train LTP was higher in mutant slices than control slices but four-train LTP was similar in both groups. Black frames indicate the time window of measurement of mean fEPSP slope (over 10 min starting 35 min after stimulation) with *P*-values. n.s., non-significant.

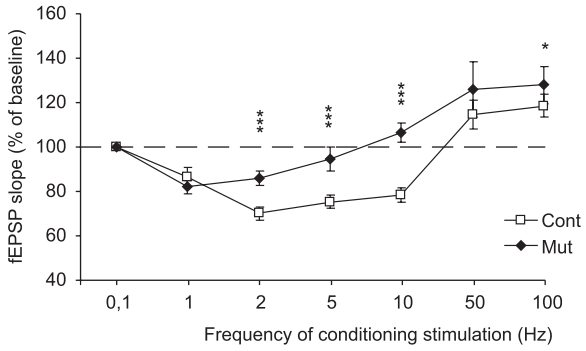

FIG. 5. Frequency-dependent enhancement in plasticity. Mean fEPSP slope measured for 10 min starting 35 min after stimulation (black frames in Figs 2–4) across the frequency range of stimulation. A clear shift of plasticity towards potentiation was observed in mutant slices. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

two trains of 100 Hz separated by 20 s, which in these conditions was similar in control and mutant slices (control, 154.1 ± 7.3% of baseline, $n = 9$ slices from three mice vs. mutant, 144.1 ± 5.4% of baseline, $n = 9$ slices from three mice, measured for 10 min 35 min after LTP induction; $F_{1,16} = 1.41$, n.s.; Fig. 6), probably due to a shorter ISI.

Depotentiation in control slices

When LFS at 1 or 2 Hz was applied 5 min after LTP induction, an almost complete reversal of LTP was observed. The residual potentiation measured for 10 min 35 min after the end of LFS was 118.3 ± 5.1% of baseline for 1 Hz ($n = 15$ slices from eight mice; $F_{1,22} = 19.39$, $P < 0.001$ vs. LTP) and 115.0 ± 10.6% of baseline for 2 Hz ($n = 11$ slices from five mice; $F_{1,18} = 9.39$, $P < 0.05$ vs. LTP; Fig. 6A, B and E). Similar results were obtained when LFS at 1 or 2 Hz was applied 30 min after LTP induction (data not shown). The residual potentiation measured for 10 min 35 min after the end of LFS was 109.6 ± 5.5% of baseline for 1 Hz [$n = 11$ slices from six mice; $F_{1,20} = 16.27$, $P < 0.001$ vs. LTP (142.3 ± 6.1% of baseline)] and 111.1 ± 9.4% of baseline for 2 Hz [$n = 8$ slices from four mice; $F_{1,14} = 4.23$, $P < 0.05$ vs. LTP (146.6 ± 13.9% of baseline).

Depotentiation in mutant slices

Unlike in control slices, 1-Hz LFS applied 5 min after LTP induction failed to depotentiate synaptic responses in mutant slices (mean residual potentiation measured for 10 min 35 min after the end of LFS: 143.3 ± 9.6% of baseline, $n = 12$ slices from seven mice; $F_{1,19} = 0.010$, n.s.; Fig. 6C and E). However, 2 Hz LFS almost fully reversed LTP (mean residual potentiation measured for 10 min 35 min after the end of LFS: 110.0 ± 7.1% of baseline ($n = 9$ slices from six mice; $F_{1,16} = 18.51$, $P < 0.001$ vs. LTP; Fig. 6D). Similar results were obtained when LFS was induced 30 min after LTP induction (data not shown). LFS at 2 Hz induced a significant LTP reversal [119.5 ± 9.4% of baseline ($n = 8$ slices from four mice); $F_{1,14} = 5.05$, $P < 0.05$ vs. LTP (140.2 ± 5.3% of baseline)] but at 1 Hz did not [129.7 ± 7.6% of baseline ($n = 7$ slices from three mice); $F_{1,12} = 1.27$, n.s. vs. LTP (140.2 ± 5.6% of baseline)].

Rescue of depotentiation by a PKA inhibitor

Because protein kinase A (PKA) can down-regulate PP1 activity through I-1 phosphorylation, we wondered whether the lack of depotentiation in the mutant slices after 1 Hz LFS could be prevented by blocking PKA. Inhibiting PKA with KT5720 (1 μM) fully reversed depotentiation induced by 1 Hz LFS 5 min after LTP induction in mutant slices. The mean residual potentiation measured for 10 min 35 min after the end of LFS was 119.7 ± 5.3% of baseline ($n = 8$ from four mice) vs. LTP values recorded in the presence of KT5720: 147.9 ± 10.0% of baseline [$n = 6$ slices from three mice; $F_{1,13} = 7.86$, $P = 0.01$ (Fig. 6F)], indicating the depotentiation impairment is PKA-dependent.

Discussion

The present results show that the partial inhibition of PP1 by genetic expression of I-1* alters the properties of bidirectional synaptic plasticity by inducing a response shift towards potentiation. PP1 inhibition does not alter LTD elicited by stimulation at 1 Hz but significantly reduces LTD elicited at 2 Hz. At 10 Hz, it favours potentiation over depression, and slightly increases LTP at 100 Hz. The shift towards potentiation is predominantly observed at intermediate frequencies, an interesting feature given the critical involvement

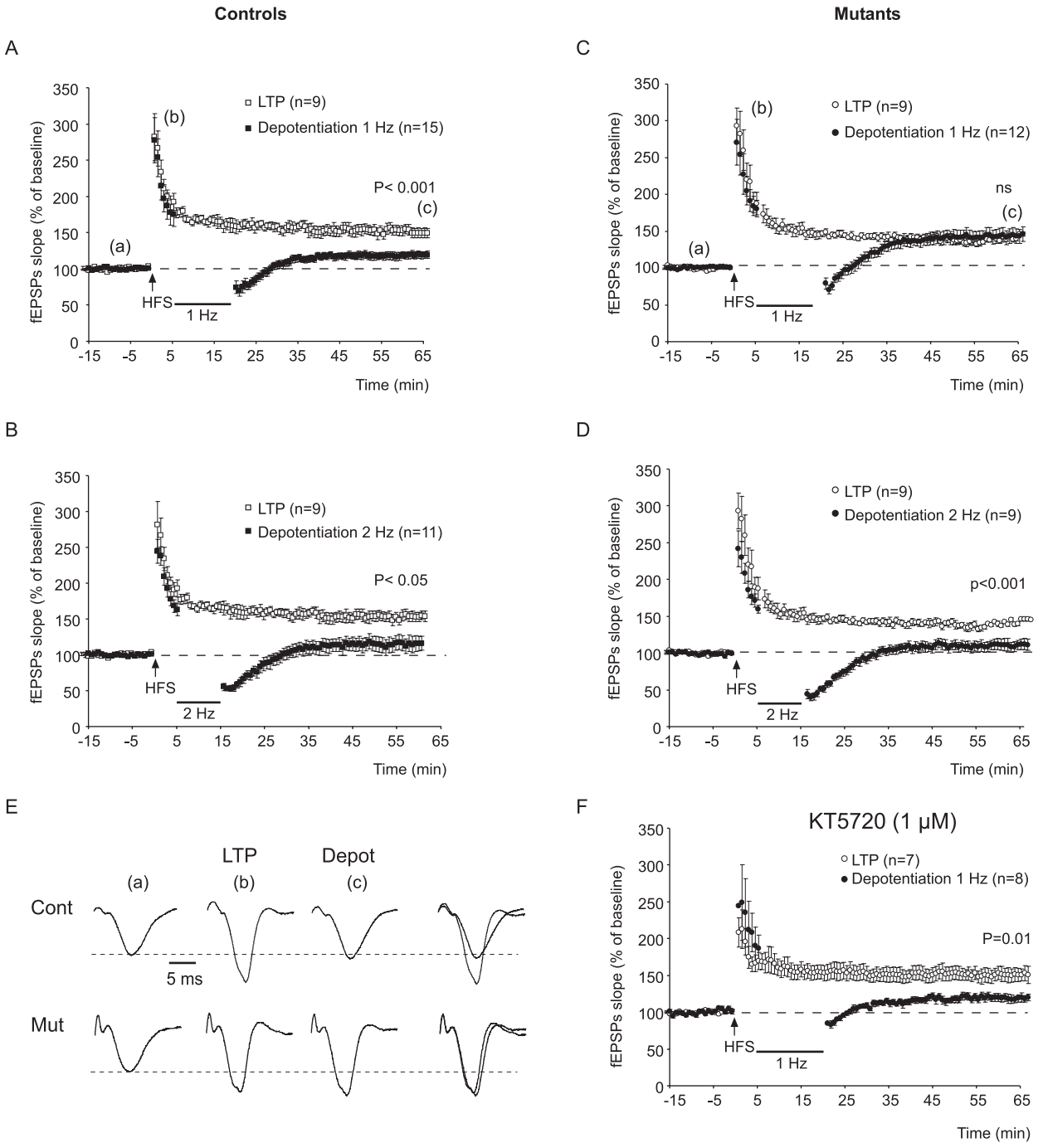

FIG. 6. Depotentiation (LTP reversal) is impaired by PP1 inhibition. Depotentiation induced by HFS (two 1-s trains at 100 Hz, 20 s ISI, open symbols; LTP) followed 5 min later by LFS at (A and C) 1 Hz for 15 min, or (B and D) 2 Hz for 10 min in control (A and B) and (C and D) mutant slices. LFS at 1 and 2 Hz after HFS induced a similar level of depotentiation in control slices (A and B, black squares). In mutant slices, only 2 Hz LFS induced depotentiation (D, black circles); no depotentiation was induced at 1 Hz (C, black circles). (E) Representative fEPSP traces before stimulation (a), after HFS (b) and after HFS followed by 1 Hz LFS (c) in control and mutant slices. (F) Depotentiation of LTP following 1 Hz stimulation in mutant slices is restored by pre-incubation of slices in the PKA inhibitor KT5720 for 2 h (black diamonds).

of neuronal activity at 4–12 Hz in cognitive tasks such as spatial learning and memory, spatial navigation or item recognition (Kahana *et al*., 2001). In turn, the frequency-dependent effect of PP1 inhibition may underlie the improved cognitive performance in the mutant mice (Genoux *et al*., 2002). Thus, the facilitated ability of mutant mice to learn the characteristics of new objects or the position of a hidden platform in a tank of water may result from the facilitated potentiation observed in the hippocampus.

The lack of effect of PP1 inhibition on 1 Hz LTD indicates that this form of LTD can be expressed even when only a small portion of PP1 activity is available (about 30% in hippocampus in I-1* mutant mice), suggesting that full PP1 activity is not required for plasticity at this stimulation frequency. PP1 is known to be required for LTD, for instance for LTD induced by 1 Hz for 5–7 min (Mulkey *et al*., 1994) or 5 Hz for 3 min (Morishita *et al*., 2001). Its sustained activity through binding to targeting partners such as neurabin or spinophilin is

necessary for the maintenance of LTD. The present results extend these findings by showing that the degree of PP1 requirement for LTD depends on the strength of stimulation. Full PP1 activation is required for 2 Hz LTD but not for 1 Hz LTD, revealing a mechanism of frequency-dependent fine-tuning of PP1 activity during LTD. This fine-tuning also operates at intermediate and high frequencies where it determines whether depression or potentiation is elicited. At 10 Hz, insufficient PP1 activity prevents synaptic depression and potentiates synaptic responses. At higher frequency, it slightly facilitates potentiation and increases LTP. Again, the effect is gradual and depends on the strength of stimulation; it is observed after one train of 100 Hz but not after four trains. These results are consistent with the observation that full expression of HFS-induced LTP is associated with endogenous inhibition of PP1 (Blitzer *et al*., 1998), and indicate that PP1 inhibition prior to stimulation facilitates potentiation, perhaps through a reduction in the threshold for LTP induction. Finally, and consistent with a general shift in favour of potentiation, depotentiation is impaired by PP1 inhibition but only with 1 Hz but not 2 Hz stimulation following LTP. This finding is in line with our previous work showing that pharmacological inhibition of PP1 prevents depotentiation (Jouvenceau *et al*., 2003) and with the hypothesis that 1- and 2-Hz stimulation differentially activate PP1. Stimulation at 2 Hz is strong enough to activate PP1 fully and overcome its inhibition by combined I-1* expression and HFS, and for resetting potentiated synapses. Together, these results highlight the existence of fine-tuned mechanisms of PP1 regulation during bidirectional plasticity.

These mechanisms may involve one or several endogenous PP1 regulators known to act either as inhibitors, targeting proteins or binding partners for substrate recognition. These regulators compete to interact with PP1 catalytic subunits and form a variety of multimeric holoenzymes dynamically modulating PP1 (Ceulemans & Bollen, 2004; Gibbons *et al*., 2005). I-1 is one PP1 inhibitor that may differentially control PP1 activity during 1- or 2-Hz LTD. It is activated by PKA-dependent phosphorylation at Thr35 and inactivated by calcineurin-dependent dephosphorylation. During stimulation at 1 Hz, it may be preferentially phosphorylated given that calcineurin activity is low, as suggested by the insensitivity of 1 Hz LTD to calcineurin inhibitors (Jouvenceau *et al*., 2003). At 2 Hz, however, calcineurin may be activated and dephosphorylate I-1, thereby relieving PP1 from inhibition. Consistent with this hypothesis, we show that the absence of depotentiation after 1 Hz stimulation is restored by a PKA inhibitor. Alternatively, it is possible that the calcineurin/I-1/PP1 complex may not be required during 1 Hz LTD as I-1 knock-out does not affect this form of LTD, at least not in dentate gyrus (Allen *et al*., 2000). In addition to I-1, the targeting proteins neurabin I and spinophilin (neurabin II) are actin-binding scaffolding proteins that control PP1 activity during plasticity. Both neurabin I/PP1 and spinophilin/PP1 complexes modulate excitatory neurotransmission by anchoring PP1 to AMPA and NMDA receptors (Feng *et al*., 2000; Terry-Lorenzo *et al*., 2005). Neurabin I/PP1 facilitates synaptic targeting of the GluR1 subunit of AMPA receptors and AMPA receptor-mediated synaptic transmission (Terry-Lorenzo *et al*., 2005), but spinophilin/PP1 down-regulates AMPA receptors (Feng *et al*., 2000). Furthermore, the spinophilin/PP1 complex contributes to LTD as the absence of spinophilin in knock-out mice displaces PP1 from its targets (prevents their dephosphorylation) and alters 1 Hz LTD in CA1 hippocampus (Feng *et al*., 2000), consistent with our results.

PP1 regulates multiple substrates in neuronal cells, several of which may be involved in the regulation of synaptic plasticity. One of these is CaMKII, which is essential for the induction of NMDA receptor-dependent forms of LTP. CaMKII activity is sustained by autophosphorylation at Thr286 (Makhinson *et al*., 1999; Colbran & Brown, 2004) and is down-regulated by dephosphorylation by PP1. Blockade of CaMKII dephosphorylation by PP1 is known to enhance the apparent co-operativity of autophosphorylation and makes CaMKII ultrasensitive to Ca^{2+} (Bradshaw *et al*., 2003). This mechanism may contribute to increase the susceptibility of synaptic circuits to potentiation in I-1* mutant slices. Indeed, CaMKII Thr286 phosphorylation was shown to be increased by I-1* expression in the mutant mice after training (Genoux *et al*., 2002). Another potential target is GluR1, which is phosphorylated on Ser845 by PKA and dephosphorylated by PP1. When phosphorylated, GluR1 is available for stable synaptic incorporation (Esteban *et al*., 2003) but is endocytotically internalized when dephosphorylated, a process that decreases the efficacy of glutamatergic transmission during LTD (Beattie *et al*., 2000; Ehlers, 2000). Interestingly, similar to CaMKII, higher Ser845 GluR1 phosphorylation was observed in I-1* mutant mice after training (Genoux *et al*., 2002), an effect that may be associated with increased AMPA receptor functioning. The NMDA receptor may also be implicated because like GluR1, NR1 is directly modulated by PP1 and PKA (Wang & Stelzer, 1994; Westphal *et al*., 1999; Scott *et al*., 2003). However, no gross change in basal glutamatergic synaptic transmission or AMPA or NMDA receptor-dependent activity was observed in mutant slices. This is in agreement with Morishita *et al*. (2001) who demonstrated that the infusion of PP1 inhibitors in neuronal cells alters synaptic plasticity but not basal synaptic currents mediated by AMPA or NMDA receptors.

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Abbreviations

AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; CaMKII, calcium/calmodulin-dependent kinase II; fEPSP, field excitatory postsynaptic potential; HFS, high-frequency stimulation; I-1, inhibitor 1; ISI, interstimulus interval; LFS, low-frequency stimulation; LTD, long-term depression, LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; PFV, presynaptic fibre volley; PKA, protein kinase A; PP1, protein phosphatase 1; PPF, paired-pulse facilitation.

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