Homeostatic plasticity induced by brief activity deprivation enhances long-term potentiation in the mature rat hippocampus

A. Félix-Oliveira,1* R. B. Dias,1,2* M. Colino-Oliveira,1,2 D. M. Rombo,1,2 and A. M. Sebastião1,2
1Instituto de Farmacologia e Neurociências, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal; and 2Unidade de Neurociências, Instituto de Medicina Molecular, Universidade de Lisboa, Lisbon, Portugal

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Félix-Oliveira A, Dias RB, Colino-Oliveira M, Rombo DM, Sebastião AM. Homeostatic plasticity induced by brief activity deprivation enhances long-term potentiation in the mature rat hippocampus. J Neurophysiol 112: 3012–3022, 2014. First published September 10, 2014; doi:10.1152/jn.00058.2014.—Different forms of plasticity occur concomitantly in the nervous system. Whereas homeostatic plasticity monitors and maintains neuronal activity within a functional range, Hebbian changes such as long-term potentiation (LTP) modify the relative strength of specific synapses after discrete changes in activity and are thought to provide the cellular basis for learning and memory. Here, we assessed whether homeostatic plasticity could influence subsequent LTP in acute hippocampal slices that had been briefly deprived of activity by blocking action potential generation and N-methyl-D-aspartate (NMDA) receptor activation for 3 h. Activity deprivation enhanced the frequency and the amplitude of spontaneous miniature excitatory postsynaptic currents and enhanced basal synaptic transmission in the absence of significant changes in intrinsic excitability. Changes in the threshold for Hebbian plasticity were evaluated by inducing LTP with stimulation protocols of increasing strength. We found that activity-deprived slices consistently showed higher LTP magnitude compared with control conditions even when using subthreshold theta-burst stimulation. Enhanced LTP in activity-deprived slices was also observed when picrotoxin was used to prevent the modulation of GABAAergic transmission. Finally, we observed that consecutive LTP inductions attained a higher magnitude of facilitation in activity-deprived slices, suggesting that the homeostatic plasticity mechanisms triggered by a brief period of neuronal silencing can both lower the threshold and raise the ceiling for Hebbian modifications. We conclude that even brief periods of altered activity are able to shape subsequent synaptic transmission and Hebbian plasticity in fully developed hippocampal circuits.

* A. Félix-Oliveira and R. B. Dias contributed equally to this work.

Address for reprint requests and other correspondence: R. B. Dias, Institute of Pharmacology and Neurosciences, Faculty of Medicine and Institute of Molecular Medicine, Univ. of Lisbon, Av. Professor Egas Moniz, Edifício Egas Moniz, 1629-028 Lisbon, Portugal (e-mail: rdias@fm.ul.pt).

Neurosciences, Faculty of Medicine and Institute of Molecular Medicine, Univ. of Lisbon, Av. Professor Egas Moniz, Edifício Egas Moniz, 1629-028 Lisbon, Portugal (e-mail: rdias@fm.ul.pt).

durability, and input specificity are the essential features that make LTP and LTD ideal cellular correlates of learning and memory formation. However, such positive feedback changes are inherently prone to generate circuit instability (Bienenstock et al. 1982). Modeling studies have shown that when left unchecked, consecutive cycles of synaptic reinforcement or weakening through LTP or LTD should, respectively, lead to runaway excitation or quiescence (Abbott and Nelson 2000; Bienenstock et al. 1982; Miller and MacKay 1994; Miller 1996) and eventually impair the ability to encode information. It is now thought that several forms of homeostatic plasticity prevent this from happening (Maffei and Fontanini 2009; Turrigiano and Nelson 2004; Vituereira and Goda 2013; Watt and Desai 2010). Homeostatic plasticity was originally described as a non-Hebbian synaptic plasticity mechanism that maintains an optimal set point of functioning by tuning overall neuronal network activity through negative feedback (Turrigiano et al. 1998; Watt and Desai 2000), regulation of neuronal excitability through intrinsic plasticity (Desai et al. 1999; Watt and Desai 2010), and adjustment of the inhibitory-excitatory balance (Kilman et al. 2002). Together, these adaptations are thought to compensate efficiently for an abnormal period of activity deprivation or increased excitability, maintaining synaptic function within a workable range. Moreover, the changes elicited by homeostatic plasticity trigger an increased availability of glutamate receptors (Lee et al. 2010; Soares et al. 2013), enhance presynaptic plasticity (Slutsky et al. 2004), and enhance intrinsic excitability (Desai et al. 1999; Sokolova and Mody 2008). All of these changes may affect the subsequent expression of Hebbian plasticity. Indeed, the probability of a specific synapse to undergo LTP or LTD is thought to reflect its previous activity history (Bienenstock et al. 1982). This homeostatic tuning of the threshold for plasticity is generally known as metaplasticity (Abraham 2008).

A few studies have directly addressed the interaction between homeostatic and Hebbian plasticity. There is an early report on the effects of dark rearing on Hebbian plasticity in the developing visual cortex (Kirkwood et al. 1996). In other brain structures, however, each form of plasticity has generally been addressed separately (reviewed in Vituereira and Goda 2013). In the hippocampus, results from two independent groups have recently suggested that homeostatic plasticity induced by chronic neuronal silencing of immature hippocampal slice cultures (Arendt et al. 2013) and cultured neurons (Lee et al. 2010) may facilitate subsequent LTP. Despite their novelty, it
remains unclear whether the priming of LTP by activity deprivation under these experimental settings mainly reflects the influence of activity deprivation on neuronal development or, alternatively, synaptic adaptation to prolonged inactivity. One way to tackle this issue is to study their interaction in an acute preparation of the mature hippocampus.

In this work, we used acute hippocampal slices to study the expression of theta-burst LTP following exposure to a brief activity deprivation protocol, previously shown to trigger homeostatic plasticity (Sutton et al. 2006). Activity deprivation was found to enhance basal synaptic transmission at the pre- and postsynaptic level without significantly affecting intrinsic neuronal excitability. When addressing the influence on LTP, we found that activity-deprived slices consistently showed higher LTP magnitude compared with control conditions even when using subthreshold theta-burst stimulation (TBS). Enhanced LTP in activity-deprived slices was also observed when picrotoxin was used to prevent the modulation of GABAergic transmission. LTP induction required the activation of NMDA receptors both under control conditions and following brief activity deprivation. Finally, we observed that consecutive LTP inductions attained a higher magnitude of facilitation in activity-deprived slices, suggesting that the homeostatic plasticity mechanisms triggered by a brief period of neuronal silencing can both lower the threshold and raise the ceiling for Hebbian modifications.

MATERIALS AND METHODS

Experiments were performed in young adult male Wistar rats (4–6 wk old) from Harlan Interfáuna Ibérica kept under standardized temperature, humidity, and lighting conditions with access to water and food ad libitum. All animal procedures were carried out according to the European Union Guidelines for Animal Care (European Union Council Directive 86/609/EEC) with the approval of the Institutional Animal Care and Use Committee. Throughout the underlying experimental work, care was taken to minimize the number of animals used.

Animals were decapitated under deep halothane anesthesia, and the brain was dissected into ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM): 124 NaCl, 3 KCl, 1.25 NaH2PO4, 26 NaHCO3, 1 MgSO4, 2 CaCl2, and 10 glucose, gassed with 95% O2-5% CO2, pH 7.4. For field excitatory postsynaptic potential (fEPSP) recordings, the hippocampus was isolated and 400-μm-thick slices were cut using a McIlwain Tissue Chopper and allowed to recover in aCSF for 1 h at room temperature. For patch-clamp recordings, acute hippocampal (300-μm-thick) slices were cut using a vibratome (VT1000 S; Leica, Nussloch, Germany) in ice-cold dissecting solution containing (in mM): 110 sucrose, 2.5 KCl, 0.5 CaCl2, 7 MgCl2, 25 NaHCO3, 1.25 NaH2PO4, and 7 glucose, gassed with 95% O2-5% CO2, pH 7.4. Slices were first incubated for 30 min at 35°C in aCSF followed by at least 1-h recovery at room temperature.

Following the recovery period, slices were separated into control and activity deprivation groups. Activity-deprived slices were incubated for 3 h with TTX (1 μM) and N-methyl-D-aspartate (NMDA; 50 μM) for 3 h, after which they recovered in artificial cerebrospinal fluid (aCSF) for 1 h before any electrophysiological study. In contrast, control slices were kept in aCSF solution but otherwise manipulated as test slices. Patch-clamp and field excitatory postsynaptic potentials (fEPSP) recordings using several stimulation protocols were performed in both groups, as described.

Recording chamber (1 ml plus 200-μl dead volume) kept at room temperature and continuously superfused at 1–2 ml/min with gassed aCSF. In both extracellular and patch-clamp recordings of afferent evoked transmission, control and activity-deprived slices were submitted to at least 15–20 min of afferent stimulation before any recordings were made to enable circuit reactivation (Gerkin et al. 2013; Sokolova and Mody 2008).

Extracellular recordings of fEPSPs. fEPSPs were recorded through an extracellular microelectrode (backfilled with 4 M NaCl, 2- to 9-MΩ resistance) placed in the stratum radiatum of the CA1 area. fEPSP data were acquired at 10 KHz using an Axoclamp-2B amplifier coupled to a DigiData 1200 interface (Axon Instruments, Foster City, CA). Each individual stimulus consisted of a 0.1-ms rectangular pulse applied once every 15 or 20 s through a concentric electrode to the Schaffer collateral fibers. Averages of six or eight consecutive responses were continuously acquired and digitized at 10 Hz with the WinLTP program (Anderson and Collingridge 2001) and quantified as the slope of the initial phase of the averaged fEPSP.

The stimulus intensity was adjusted at the beginning of the experiments to obtain a fEPSP slope close to 0.5 mV/ms. For input-output curves, after a stabilization period under the standard stimulation conditions, the stimulus intensity was increased by 20 μA every 6 min (60–560 μA). Paired-pulse facilitation (PPF) was quantified as the percentage of slope facilitation between two consecutive fEPSPs (fEPSP2/fEPSP1) elicited with a 50-ms interstimulus interval. For LTP induction, four different stimulation protocols were used. The weakest protocol consisted of one train containing one burst with four pulses at 100 Hz [1 × (1 × 4)]. The three remaining protocols were theta-burst variations of this single-stimulation train where a burst always consisted of four pulses delivered at 100 Hz, each burst within a train was separated by a 200-ms interval (i.e., 5-Hz burst frequency),
and each train was separated by a 10-s interval. We used 1 train containing 2 bursts \([1 \times (2 \times 4)]\), 1 train containing 3 bursts \([1 \times (3 \times 4)]\), and 3 consecutive trains each containing 15 bursts \([3 \times (15 \times 4);\) Chen et al. 1999a; Fontinha et al. 2008\]. The magnitude of LTP was assessed by measuring the average fEPSP slope facilitation obtained 50–60 min after the induction normalized to a 10-min baseline period. In some experiments, LTP was induced in the presence of the selective NMDA receptor antagonist d-APV (200 \(\mu\)M; Ericksson et al. 2010) or the GABA\(_\text{A}\) receptor antagonist picrotoxin (50 \(\mu\)M; Chen et al. 1999b). Each drug was present for at least 30 min before LTP induction. Posttetanic potentiation (PTP) was assessed as the average fEPSP slope obtained in the 1st 8 min after LTP induction (Habets and Borst 2007).

**Patch-clamp recordings.** Whole cell patch-clamp recordings were obtained from CA1 pyramidal cells visualized with an upright microscope (Zeiss Axioskop 2FS) equipped with infrared video microscopy and differential interference contrast optics. Recordings were performed at room temperature in current-clamp or voltage-clamp mode [holding potential \(V_h = -70\) mV] with an Axopatch 200B (Axon Instruments) amplifier. To record excitatory synaptic currents, patch pipettes (4- to 7-M\()\) resistance) were filled with an internal solution containing (in mM): 125 K-gluconate, 11 KCl, 0.1 CaCl\(_2\), 2 MgCl\(_2\), 1 EGTA, 10 HEPES, 10 glucose, 5 MgATP, and 0.4 NaGTP, pH 7.2, adjusted with CsOH (50 wt% in H\(_2\)O), 280–290 mosM. Acquired signals were filtered using an in-built, 2-kHz, three-pole Bessel filter, and data were digitized at 5 kHz under control of the pCLAMP 10 (Molecular Devices) software program. The junction potential was not compensated for, and offset potentials were nulled before gigaseal formation.

The resting membrane potential was measured immediately on establishing whole cell configuration. For each neuron, the threshold for action potential generation was determined as the difference between the resting membrane potential and the membrane potential at which phase plot slope reached 10 mV/ms (Naundorf et al. 2005). To assess PPF, afferent-evoked excitatory postsynaptic current (EPSCs) were elicited by paired 0.2-ms rectangular pulses delivered 50 ms apart through a concentric electrode (Harvard) placed in the Schaffer collaterals/commissural afferents to the CA1 area. EPSC recordings were performed in the presence of the GABA\(_\text{A}\) receptor blocker gabazine (2 \(\mu\)M). Paired responses were evoked every 15 s, and averages of four consecutive individual recordings were used for analysis. PPF was quantified as the percentage of amplitude facilitation between EPSC2 and EPSC1. Offline analysis of action potential and PPF data was performed using the Clampfit 10.2 software.

Spontaneous miniature excitatory postsynaptic currents (mEPSCs) were elicited by paired 0.2-ms rectangular pulses delivered 50 ms apart through a concentric electrode (Harvard) placed in the Schaffer collaterals/commissural afferents to the CA1 area. mEPSC recordings were performed in aCSF supplemented with TTX (50 \(\mu\)M) and gabazine (2 \(\mu\)M). Miniature inhibitory postsynaptic currents were recorded in aCSF supplemented

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**Fig. 2.** Brief activity deprivation (AD) drives synaptic scaling of spontaneous synaptic transmission. Compared with control conditions (CT), blockade of action potentials (AP) and N-methyl-d-aspartate (NMDA) receptors with TTX (1 \(\mu\)M) and d-APV (50 \(\mu\)M) for 3 h, followed by 1 h of recovery, significantly increases the amplitude (A) and frequency (B) of miniature excitatory postsynaptic currents (mEPSCs) while decreasing their average decay time (C; \(n = 7–8\)). Representative mEPSC tracings are shown for each condition (D). In contrast, brief activity deprivation does not affect the average amplitude (E) of miniature inhibitory postsynaptic currents (mIPSCs) but significantly decreases the frequency (F) with which they occur (\(n = 7\) per condition). Representative mIPSC tracings are shown in G. \(^{*}\)\(P > 0.05;\) \(^{*}\)\(P < 0.05\) (unpaired \(t\)-test). n.s., Not significant.

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with TTX (500 nM) and kynurenic acid (1 mM). Analysis of miniature events was performed using the Synaptosoft Mini Analysis Program software with the amplitude threshold for event detection being set at 3× the average root-mean-square noise.

Drugs and stock solutions. TTX (Abcam), dl-APV (Abcam), and gabazine (Ascent) were prepared in distilled water as 1, 50, and 10 mM stock solutions, respectively. Picrotoxin (Ascent) was prepared in dimethyl sulfoxide as a 50 mM stock solution, and kynurenic acid (Abcam) was prepared in distilled water as a 100 mM stock solution.

Statistical analysis. Data are expressed as means ± SE from n slices (fEPSP recordings) or n neurons (patch-clamp recordings). The normal distribution of the data was assessed by the Kolmogorov-Smirnov test, and data homoscedasticity was assessed with the F test of equality of variances. Mean differences between groups were evaluated by two-tailed unpaired t-test. The Mann-Whitney test was applied to nonnormally distributed samples. Unpaired t-tests with Welch correction were applied to groups with unequal variances. Comparisons between fEPSP slopes before (baseline) and after TBS were performed with the paired Student’s t-test. Values of P < 0.05 were considered to account for statistically significant differences.

RESULTS

Brief activity deprivation enhances spontaneous and afferent-evoked synaptic transmission. To evaluate whether a 3-h period of global activity deprivation was enough to drive homeostatic plasticity, we first studied its effects on spontaneous synaptic transmission. In keeping with the initial experiments performed by Sutton et al. (2006), we found that brief activity deprivation was associated with a significant increase in the average amplitude of mEPSCs as well as in the frequency with which they occurred (n = 7–8; Fig. 2, A–D), indicating a scaling up of spontaneous excitatory synaptic transmission through pre- and postsynaptic modifications. We further observed a significant decrease in the frequency of spontaneous inhibitory events (n = 7 per condition; Fig. 2, E–G) suggesting that an attenuation in the inhibitory inputs to pyramidal neurons could also contribute for a scaling up of excitatory synaptic transmission.

In addition to upregulation of postsynaptic glutamate receptors and presynaptic neurotransmitter release, homeostatic plasticity may occur through regulation of intrinsic neuronal excitability (Desai et al. 1999; Turrigiano and Nelson 2004). Indeed, prolonged (48-h) activity deprivation is known to affect intrinsic excitability as a result of modulation of ionic channel number and/or activity (Desai et al. 1999). To address possible changes in intrinsic excitability after brief (3-h) activity deprivation, we next performed whole cell patch-clamp recordings under current-clamp mode from control and activity-deprived neurons. No significant differences (P > 0.05, unpaired t-test) were observed in the resting membrane potential (−60.3 ± 1.2 mV in control and −59.1 ± 1.5 mV in activity-deprived neurons; Fig. 3A), action potential threshold from resting potential (10.7 ± 1.7 mV in control and 13.4 ± 1.9 mV in activity-deprived neurons; Fig. 3, B and C), instantaneous firing frequency to injected current relationship (Fig. 3D), or the maximum firing frequency (24 ± 3.2 Hz in control and 22 ± 1.4 Hz in activity-deprived neurons; Fig. 3, E and F; n = 6–11 per condition). Average membrane resistance (154 ± 10 MΩ for control and 150 ± 11 MΩ for activity-deprived neurons) and access resistance (28.7 ± 2.3 MΩ for control and 28.8 ± 1.6 MΩ for activity-deprived neurons) were also comparable between experimental conditions (P > 0.05, unpaired t-test).

Input-output curves were also obtained to characterize basal synaptic transmission changes in activity-deprived slices. In Fig. 3, Brief activity deprivation does not alter the resting membrane potential (RMP) nor the firing threshold of CA1 pyramidal neurons. RMP were measured immediately on establishing whole cell configuration and did not significantly differ between control and activity-deprived neurons (A). Changes in membrane potential and AP were evoked under current-clamp mode by injection of 500-ms current pulses (−25 to +275 pA in 12.5- or 25-pA increments) from an initial holding potential (Vh) of −70 mV. The threshold for AP generation was measured as the beginning of the upward rise of the AP when dV/dt exceeded 10 mV/ ms. The delta between the threshold potential of each neuron and the Vh at which they 1st fired an AP was not significantly different between conditions (B). Representative tracings for each condition are shown in C. Instantaneous AP firing frequency to injected current plots were comparable between conditions, as was the maximum AP frequency attained (D–F; n = 6–11 per condition; **P > 0.05, unpaired t-test).
line with the ability for homeostatic plasticity to drive increased synaptic efficiency (Arendt et al. 2013; Turrigiano and Nelson 2004), we observed that the extracellular responses to afferent stimulation were increased in activity-deprived slices. Indeed, the maximum fEPSP slope, defined as the response to the strongest stimulus used, was higher in slices submitted to activity deprivation (-2.72 ± 0.24 mV/ms) compared with control slices (-1.79 ± 0.21 mV/ms; n = 8; P < 0.05, unpaired t-test; Fig. 4A). The presynaptic fiber volley (PSFV) amplitude was not significantly different between control and activity-deprived slices, as also demonstrated by the PSFV amplitude elicited with the strongest stimulus (1.11 ± 0.13 mV in control and 1.39 ± 0.22 mV in activity-deprived slices; Fig. 4B), suggesting an increase in synaptic efficiency (Fig. 4C) in the absence of major changes in the recruitment of presynaptic neurons (n = 8 per condition; P > 0.05, unpaired t-test). Taken together, these findings suggest that, under our experimental conditions, brief activity deprivation triggers homeostatic changes in synaptic transmission in the absence of gross modifications in neuronal excitability.

Brief activity deprivation affects both short- and long-term synaptic plasticity. Having established that brief activity deprivation was able to upregulate both spontaneous and afferent-evoked excitatory synaptic transmission, we next wanted to address its effects on subsequent synaptic plasticity. Prolonged (60-h) blockade of evoked glutamate release in cultured slices was recently shown to enhance the ability of the hippocampal circuitry to undergo pairing-induced LTP through silent synapse formation (Arendt et al. 2013). However, application of the same activity deprivation model to cultured hippocampal slices has also been shown to trigger seizure-like activity (Trasande and Ramirez 2007) suggestive of maladaptive network reorganization (Fröhlich et al. 2008). Here, we used acute slices and brief blockade of neuronal activity to address the interaction between homeostatic and Hebbian plasticity induced by TBS in a mature neuronal circuit.

We first studied LTP by delivering 1 × (3 × 4) TBS to control and activity-deprived slices (see MATERIALS AND METHODS). With this protocol, LTP magnitude was 126.5 ± 4.1% of baseline fEPSP under control conditions (n = 8). In contrast, when applied to activity-deprived slices, the same induction protocol produced significantly higher fEPSP facilitation (LTP magnitude: 146.0 ± 7.7%; n = 7; P < 0.05, unpaired t-test; Fig. 5, A and B), suggesting that even a brief period of activity deprivation is able to enhance subsequent Hebbian plasticity. Accompanying this enhancement in LTP, brief activity deprivation also increased PTP (Fig. 5C). This form of short-term plasticity is thought to reflect the increase in neurotransmitter release that arises from residual Ca2+ accumulating in the presynaptic terminals during the trains of high-frequency stimulation, leading to PKC activation and enhanced vesicle fusion (Korogod et al. 2007; Zucker and Regehr 2002). Indeed, PTP was significantly increased after activity deprivation (180.4 ± 15.3% of baseline fEPSP slope; n = 7) compared with that observed under control conditions (139.3 ± 4.4% of baseline fEPSP slope; n = 8; P < 0.05, unpaired t-test with Welch correction).

We also evaluated the repercussions of brief activity deprivation on a second form of short-term presynaptic plasticity. Changes in the release probability of glutamate at the Schaffer collateral-CA1 synapse were assessed through PPF of the fEPSP slope (McNaughton 1982). We found that PPF was significantly higher in control conditions (158 ± 5.33% of fEPSP slope facilitation) compared with slices that had been submitted to activity deprivation (143 ± 4.32% of fEPSP slope facilitation), which is compatible with enhanced basal presynaptic function in activity-deprived slices (n = 11; P < 0.05, unpaired t-test; Fig. 5D). Similar results were observed when whole cell responses to paired afferent stimulation were recorded in the presence of gabazine (2 μM) at a Vm of -70 mV. Under control conditions, there was an average 211 ± 21.8% EPSC amplitude facilitation by delivery of paired pulses (n = 6) vs. 157 ± 9.39% EPSC amplitude facilitation (n = 8) in activity-deprived slices (P < 0.05, unpaired t-test; Fig. 5, E and F).

Fig. 4. Increased basal synaptic transmission following activity deprivation in acute hippocampal slices. Input-output curves are from control (C; n = 8) and activity-deprived slices ( ); where the fEPSP slope (A) or the presynaptic fiber volley (PSFV) amplitude (B) are plotted as a function of stimulus intensity (60–360 μA). As shown in A, right, the maximum fEPSP slope, defined as the average fEPSP slope response to the strongest stimulus used, was significantly higher in activity-deprived slices. B, right shows that the maximum PSFV amplitude, measured as the averaged PSFV amplitude obtained with the highest stimulus, was not significantly different between conditions. In C, the fEPSP slope is plotted against the PSFV amplitude. *P < 0.05; **P < 0.05, unpaired t-test.
Increased LTP after activity deprivation is dependent on NMDA receptor activation and does not arise from decreased GABAergic transmission. Enhanced LTP following brief activity deprivation could arise not only from increased availability of postsynaptic glutamate receptors or optimized glutamate release, but also from the engagement of LTP induction pathways partially or completely independent of NMDA receptors. To determine whether the increased LTP magnitude following activity deprivation was dependent on NMDA receptor activation, we compared the effect of a 1 × (3 × 4) theta-burst stimulation (TBS) protocol in control and activity-deprived slices. Under control conditions, LTP magnitude was 121.7 ± 3.4% of baseline values (n = 5), whereas in activity-deprived slices, LTP magnitude reached 159.0 ± 10.7% of baseline values (n = 5; P < 0.05, unpaired t-test; Fig. 6, C and D). Thus the increased LTP observed following brief activity deprivation does not seem to be due to an adaptive downregulation of inhibitory transmission in activity-deprived slices compared with control conditions.
Fig. 6. Enhanced LTP following brief activity deprivation is NMDA receptor-dependent. A: time course of changes in fEPSP slope after stimulation at $t = 0$ min with $1 \times (3 \times 4)$ TBS in control (n = 5) and activity-deprived slices (n = 6) in the presence of DL-APV (200 µM). B: for each condition, NMDA receptor blockade largely prevents LTP. C: the same TBS protocol was delivered to control (n = 5) and activity-deprived slices (n = 5) in the presence of picrotoxin (50 µM) to cancel the GABAergic component of synaptic transmission. D: note that under these conditions, LTP was still significantly greater in activity-deprived slices. *P < 0.05; **P < 0.01, unpaired t-test.

The ceiling for LTP is raised after brief activity deprivation. Mechanistically, enhanced LTP following brief activity deprivation could arise from an increased ability of hippocampal synapses to undergo theta-burst LTP, expanding the ceiling for plasticity in a mature ex vivo structure. To assess this hypothesis, we performed LTP saturation experiments that consisted of three consecutive LTP inductions, interspaced by 60 min, using $1 \times (3 \times 4)$ or stronger $3 \times (15 \times 4)$ TBS (see MATERIALS AND METHODS).

In line with the first set of LTP experiments described above (Fig. 5), the first LTP (LTP1) induced with the $1 \times (3 \times 4)$ TBS had an amplitude of $149.3 \pm 4.5\%$ of baseline values in activity-deprived slices, being significantly higher than that observed in control conditions ($122.2 \pm 4.7\%$ of baseline fEPSP slope; n = 8 per condition; P < 0.05, unpaired t-test). This difference was maintained throughout the second consecutive LTP (LTP2: $137.6 \pm 5.4\%$ in control conditions vs. $164.0 \pm 5.2\%$ following activity deprivation; n = 8; P < 0.05, unpaired t-test) and the third LTP (LTP3: $144.8 \pm 4.6\%$ in control conditions and $170.8 \pm 9.4\%$ following activity deprivation; n = 8; P < 0.05, unpaired t-test; Fig. 7, A and B). Similar results were also observed with the stronger $3 \times (15 \times 4)$ TBS, which elicited higher LTP after activity deprivation compared with control conditions, for LTP1 ($130.0 \pm 4.2\%$ for control conditions and $184.1 \pm 9.7\%$ for activity-deprived slices), LTP2 ($141.4 \pm 10.5\%$ comparing with $201.9 \pm 10.2\%$), and LTP3 ($146.8 \pm 9.5\%$ comparing with $206.8 \pm 9.9\%$; n = 5 per condition; P < 0.05 for each LTP, unpaired t-test; Fig. 7, D and E). The behavior of control and activity-deprived slices is similar for LTP2 and LTP3, with the difference arising from the enhanced LTP already attained in LTP1. In fact, with the $3 \times (15 \times 4)$ TBS, the LTP3-to-LTP1 ratio was 1.13 ± 0.07 in control conditions and 1.12 ± 0.04 in activity-deprived slices (P > 0.05, unpaired t-test).

Subthreshold TBS induces LTP in activity-deprived slices. We next evaluated the effects of two weaker LTP protocols, namely $1 \times (1 \times 4)$ and $1 \times (2 \times 4)$ TBS (see MATERIALS AND METHODS), to address the possible occurrence of changes in the threshold for LTP induction. For each condition, we compared fEPSP slope values at the end of LTP or PTP with the fEPSP slope values recorded before applying a TBS (during the baseline period) to evaluate whether a determined stimulus was able to alter synaptic transmission significantly. Using this analysis, no significant changes in synaptic transmission were observed under control conditions in response to $1 \times (1 \times 4)$ stimulation (average fEPSP slope 50–60 min after train delivery: $102.3 \pm 2.6\%$ of baseline values; n = 4; P > 0.05, paired t-test). When applied to activity-deprived slices, the same $1 \times (1 \times 4)$ protocol induced a significant facilitation of synaptic transmission (124.4 ± 5.4% of baseline fEPSP slope; n = 5, P < 0.05, paired t-test). With the $1 \times (2 \times 4)$ TBS, a small but nonsignificant facilitation of synaptic transmission was observed under control conditions (107.1 ± 6.2%; n = 4; P > 0.05, paired t-test), whereas a significant LTP was obtained in activity-deprived slices (129.3 ± 3.2%; n = 5; P < 0.05, paired t-test). Figure 8 illustrates the effect of increasing theta-burst protocol strength on LTP magnitude for control and activity-deprived slices, summarizing the results obtained with the different LTP protocols used throughout the study. Changes in PTP are also illustrated for the various stimulation protocols.
used. For each protocol, LTP and PTP magnitudes were consistently higher in activity-deprived slices.

DISCUSSION

Taken together, our data support the ability for a discrete period of activity deprivation to regulate basal synaptic transmission and decrease the threshold for LTP induction, lowering the requirements for Hebbian plasticity. Additionally, brief activity deprivation seems to increase the extent to which mature hippocampal synapses can undergo Hebbian modifications by raising the ceiling for theta-burst LTP.

In vivo, physiological interactions between homeostatic and Hebbian forms of plasticity are likely to occur through development, learning, memory formation, and consolidation during sleep. Also, pathological stimuli such as deafferentation, traumatic brain injury, and stroke may trigger homeostatic plasticity as a response to input loss and decreased neuronal activity levels. The resulting hyperexcitability could potentially facilitate recovery from injury by promoting new synapse formation (Murphy and Corbett 2009) and, as we now show, by influencing the dynamics of subsequent Hebbian plasticity phenomena. However, recovery may come at a cost. Overcompensatory effects have been associated with the generation of reverberant circuits and postslesional epileptogenesis (Frohlich et al. 2008; Trasande and Ramirez 2007; Queenan and Pak 2013). Indeed, a decrease in the threshold for Hebbian forms of plasticity may likely contribute to reinforce reverberant circuits and epileptogenesis after synapse silencing.

In this study, activity deprivation was induced by blocking action potential generation with TTX for 3 h while simultaneously preventing activation of NMDA receptors by spontaneously released glutamate. In previous studies and since the original work by Turrigiano et al. (1998), prolonged activity deprivation protocols with 48-h exposure to TTX have been used to characterize homeostatic plasticity. An important limitation to these protocols is that they are only applicable to cultured neurons and organotypic slices, both of which are still undergoing neuronal differentiation and synapse maturation. Although the process of slice preparation is not devoid of collateral damage itself, acute hippocampal slices are commonly accepted to largely preserve intrinsic neuronal circuitry (Lossi et al. 2009). Under our experimental conditions, brief activity deprivation led to an enhancement in the excitatory component of spontaneous synaptic transmission and a converse reduction in inhibitory minis onto CA1 pyramidal neurons in the absence of gross modifications in neuronal excitability. Even though the mEPSC data largely agree with those of Sutton et al. (2006), we observed an additional increase in event frequency. This difference may rely on experimental animal age variations, known to affect the site of homeostatic plasticity changes (Pozo and Goda 2010), but it may also result from more events being detected as unitary postsynaptic responses become larger in activity-deprived neurons (Ito and Schuman 2009). In addition, we also observed a significant decrease in miniature inhibitory transmission that had not been previously described by Sutton et al. (2006). Our results are in...
line with reports of complementary changes in inhibitory transmission opposite to those occurring at excitatory synapses as a result of homeostatic plasticity (Wenner 2011). Removal of synaptic inhibition onto restricted areas of CA1 dendritic trees has been previously shown to selectively prime nearby excitatory synapses and facilitate subsequent LTP induction (Chevaleyre and Castillo 2004). Under our experimental conditions, picrotoxin was not able to prevent LTP enhancement in activity-deprived slices, therefore excluding the possibility that changes in inhibitory transmission per se could fully account for the changes in LTP after activity deprivation. Further work is, however, still required to clarify the implications of the changes in spontaneous inhibitory miniature transmission observed after activity deprivation.

In contrast to previous work using prolonged activity deprivation in cultured primary visual cortical neurons (Desai et al. 1999), we have not observed significant effects on intrinsic neuronal excitability. This suggests that this brief activity deprivation model may predominantly trigger compensatory synaptic modifications. Additional recruitment of intrinsic plasticity may require longer periods of deprivation or may depend on the developmental stage, experimental model, and brain area studied. It should also be noted that our experimental design does not exclude possible alterations in the integration of dendritic signals and their conduction to the neuronal soma.

In addition to the study of miniature excitatory synaptic transmission as performed in the initial validation of the model (Sutton et al. 2006), we evaluated the effects of brief activity deprivation on evoked population responses, which were consistently enhanced compared with control conditions. Enhanced excitatory synaptic transmission may reflect the up-regulation of postsynaptic AMPA receptors, widely recognized as major mediators of homeostatic plasticity (O’Brien et al. 1998; Sutton et al. 2006; Turrigiano et al. 1998). Compensatory scaling up of postsynaptic AMPA receptors has been shown to occur following even shorter suppression of NMDA receptor activation (Nosyreva et al. 2013). Other possible contributory mechanisms for homeostatic plasticity include increases in vesicle pool size and the release probability of individual neurotransmitter vesicles (Murthy et al. 2001; Slutsky et al. 2004), but, most likely, coordinated pre- and postsynaptic modifications take place.

Having established that brief neuronal silencing was enough to enhance both spontaneous and afferent-evoked transmission, that is to say, to induce homeostatic plasticity in acute slices, we investigated whether it could also subsequently influence Hebbian forms of synaptic plasticity. Both short- and long-term forms of synaptic plasticity were altered as a result of brief activity deprivation. PPF results are compatible with enhanced basal glutamate release in activity-deprived slices (but see Opazo et al. 2010 for an alternative postsynaptic mechanism underlying PPF) and suggest that brief activity deprivation may trigger presynaptic optimization of neurotransmitter release (Murthy et al. 2001; Slutsky et al. 2004) in addition to the changes in the postsynaptic compartment initially described by Sutton et al. (2006). These changes must encompass some of the mechanisms by which prior activity deprivation affects the ability of hippocampal synapses to undergo Hebbian plasticity subsequently, but additional modifications are most probably also required.

As we now show, following 3 h of activity deprivation, LTP induction is still dependent on NMDA receptor activation, but the magnitude of LTP becomes higher, suggesting that even a discrete period of neuronal silencing provides an appropriate stimulus for metaplasticity. As first defined (Abraham and Bear 1996), the concept of metaplasticity hypothesizes that the propensity for synapses to express LTP reflects their past history (Abraham 2008; Abraham and Bear 1996). One of the ways to prime plasticity is to lower the threshold for sustained changes in synaptic transmission (Savić et al. 2003), which is in keeping with the notion that different hippocampal synapses display distinct thresholds for LTP (Lynch et al. 2012). An association between visual deprivation and a diminished

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**Fig. 8.** Decreased threshold for short-term potentiation and LTP in activity-deprived slices. **A:** comparison of the facilitation obtained with the different TBS protocols is shown for control (○; n = 4–8) and activity-deprived (●; n = 5–7) slices. **B:** for the same experiments, PTP values were also compared between control (○) and activity-deprived (●) slices. Data plotted for the 1 × (3 × 4) and the 3 × (15 × 4) TBS protocols correspond to results shown in Figs. 5 and 7, respectively. Note that the 1 × (1 × 4) train was unable to produce a significant LTP of synaptic transmission under control conditions but induced LTP in activity-deprived slices. In turn, the 1 × (2 × 4) TBS induced a small but nonsignificant facilitation of fEPSPs under control conditions but a significant LTP in activity-deprived slices. For each group of experiments, comparison with the baseline was performed using the paired t-test (*P < 0.05); the difference between experimental conditions was assessed using unpaired t-tests (**P < 0.05) or the Mann-Whitney for nonnormally distributed samples (αP < 0.05).
threshold for plasticity in the visual cortex was proposed even before the concept of homeostatic plasticity was established. Kirkwood et al. (1996) showed that two days of dark rearing resulted in a lowered threshold for LTP in the rat visual cortex, which was rapidly reverted by exposure to light. Dark rearing from birth has also been shown to maintain high plasticity levels at the visual cortex (Mower et al. 1983) in line with later work showing that brief monocular deprivation can reactivate ocular dominance plasticity in the adult visual cortex (He et al. 2006). The fact that the adult visual cortex can restore its ability to express functional ocular dominance plasticity in response to experience (Pham et al. 2004; Tagawa et al. 2005) may reflect a form of metaplasticity (Sawtell et al. 2003). As our data now suggest, such an interaction between inactivity and plasticity may also occur in other brain areas, namely the hippocampus, within a few hours of depriving mature synapses from activity. Future work is required to clarify the signaling mechanisms underlying such metaplasticity. Interesting hypotheses include the permissive role of previous rounds of TBS on the diffusion of factors enabling subsequent potentiation of neighboring synapses (Kramár et al. 2012). Particularly, brain-derived neurotrophic factor is known to mediate heterosynaptic priming of plasticity through synaptic tagging and capture (Sajikumar and Korte 2011).

In addition to changes in threshold, we also hypothesized that enhanced LTP could reflect an increase in the LTP ceiling, since activity deprivation is known to induce silent synapse formation (Arendt et al. 2013; Nakayama et al. 2005). Changes in the ceiling for synaptic modification through LTP have already been described in the motor cortex as a function of prior experience (Rioult-Pedotti et al. 2000). We therefore evaluated the consequences of successive stimulation protocols with similar or different strengths and found that brief activity deprivation lowered the requirements for afferent activity to induce LTP as well as seemed to raise the plateau of facilitation attained in LTP saturation experiments. We therefore showed that it is possible to expand the dynamic range for plasticity expression under particular conditions, which may allow maximizing the distribution of resources in response to extreme variations of overall activity levels. These may for example occur during ischemia, which prompts a delayed facilitation of synaptic transmission involving calcium-permeable AMPA receptors and modulation by adenosine (Dias et al. 2013b), a ubiquitous retaliatory metabolite well-fitted to adjust neuronal activity to available energy stores (Dias et al. 2013a). Homeostatic plasticity triggered by neuronal activity dampening in stroke may promote the formation of new connections between surviving neuronal cells, later pruned by means of Hebbian plasticity to refine neuronal circuits and potentially recover some function (Murphy and Corbett 2009).

In conclusion, our data clearly show that brief activity deprivation is not only able to enhance excitatory synaptic transmission, but also may prime Hebbian plasticity by lowering the threshold and raising the ceiling for LTP in acute hippocampal slices. Hence, activity deprivation may represent a stimulus for metaplasticity of potential relevance under both physiological and pathological conditions.

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