Switching off LTP: mGlu and NMDA Receptor–Dependent Novelty Exploration–Induced Depotentiation in the Rat Hippocampus

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Abstract

Both electrically induced synaptic long-term potentiation (LTP) and long-term depression have been extensively studied as models of the cellular basis of learning and memory mechanisms. Recently, considerable interest has been generated by the possibility that the activity-dependent persistent reversal of previously established synaptic LTP (depotentiation) may play a role in the time- and state-dependent erasure of memory. Here, we examined the requirement for glutamate receptor activation in experience-induced reversal of previously established LTP in the CA1 area of the hippocampus of freely behaving rats. Continuous exploration of non-aversive novelty for ~30 min, which was associated with hippocampal activation as measured by increased theta power in the electroencephalogram, triggered a rapid and persistent reversal of high frequency stimulation-induced LTP both at apical and basal synapses. Blockade of metabotropic glutamate (mGlu) receptors with mGlu5 subtype–selective antagonists, or N-methyl-D-aspartate (NMDA) receptors with GluN2B subunit–selective antagonists, prevented novelty-induced depotentiation. These findings strongly indicate that activation of both mGlu5 receptors and GluN2B-containing NMDA receptors is required for experience-triggered induction of depotentiation at CA3-CA1 synapses. The mechanistic concordance of the present and previous studies of experience-induced and electrically induced synaptic depotentiation helps to integrate our understanding of the neurophysiological underpinnings of learning and memory.

Introduction

Persistent increases in synaptic strength in the hippocampus during the acquisition of certain behavioral tasks share mechanisms with high frequency stimulation-induced long-term potentiation (LTP) (Gruart et al. 2006; Whitlock et al. 2006; Madronal et al. 2007). These forms of electrically and behaviorally induced persistent potentiation of transmission at synapses from CA3 onto CA1 (CA3-CA1) pyramidal cells can occlude each other, consistent with learning-related changes in synaptic weights being distributed widely across the dorsal hippocampus. Difficulties in detecting the predicted LTP-like synaptic strengthening during acquisition of other hippocampal-dependent tasks may be attributable to the parallel engagement of other forms of synaptic plasticity, such as long-term depression (LTD), depotentiation, and synaptic scaling (Neves et al. 2008).

Indeed, novelty acquisition can induce a time-dependent persistent reversal of previously established LTP in the hippocampus (Xu et al. 1998; Manahan-Vaughan and Braunewell 1999; Abraham et al. 2002; Straube et al. 2003; Collingridge et al. 2010). Thus, exploration of a novel environment can trigger a rapid depotentiation of LTP at CA3-CA1 synapses induced by high-frequency conditioning stimulation (HFS) that is prevented by prior acquisition of information about the new environment. Furthermore, prior induction of depotentiation by novelty occludes a novelty-facilitated induction of LTD by low-frequency stimulation (LFS) (Kemp and Manahan-Vaughan 2004).

Recently, great interest has been generated in the discovery of a similar experience-dependent persistent reversal of behaviorally induced LTP-like changes in the amygdala. Here, cued fear-conditioning induces widespread synaptic strengthening (McKernan and Shinnick-Gallagher 1997; Rogan et al. 1997; Rumpel et al. 2005), and there is growing evidence that fear memories are transiently susceptible to erasure due to the ability of additional experience to reverse this form of synaptic potentiation (Kim et al. 2007; Clem and Huganir 2010; Diaz-Mataix et al. 2011). A similar role of depotentiation in hippocampal-dependent memory suppression is now gaining support (Zhang et al. 2011).

The receptor mechanisms underlying the induction of depotentiation by novelty exploration in the hippocampus are not known. One likely possibility is that it shares mechanisms with LFS-induced time-dependent reversal of LTP (Staubli and Lynch 1990; Fujii et al. 1991; Bashir and Collingridge 1994) and LTD. Depending on experimental conditions, the induction of both LTD and depotentiation by LFS at CA3-CA1 synapses have been reported to require the activation of N-methyl-d-aspartate (NMDA) and metabotropic glutamate receptors (mGluRs), even though the sites of expression of LTD and depotentiation often differ (Collingridge et al. 2010; Luscher and Huber 2010; Popkirov and Manahan-Vaughan 2011). Of particular note, a selective role for LTD-like synaptic plasticity in mediating hippocampal-dependent memory consolidation was posited based on the requirement for NMDA receptors (NMDARs) containing GluN2B subunits (Ge et al. 2010).

Here, we examined the requirement for different glutamate receptors in novelty-induced depotentiation. Prolonged, but not brief, novelty exploration triggered depotentiation. The depotentiation was widespread, reversing LTP both at apical and basal synaptic inputs from CA3 to CA1. Both mGluRs, in particular mGlu5 subtype, and NMDARs, in particular subtypes containing GluN2B subunits, were required for the induction of depotentiation by novelty exploration. The present findings support a role for NMDAR- and mGluR-dependent synaptic depotentiation in enabling the storage of new information by the hippocampus.

Materials and Methods

Electrode Implantation and Electrophysiology

Experiments were performed on freely behaving male Wistar rats, weighing 300–350 g under license from the Department of Health, Ireland. Implantation of electrodes in the stratum radiatum of the CA1 region was carried out under anesthesia using a mixture of ketamine (80 mg/kg) and xylazine (8 mg/kg) (both intraperitoneal [i.p.]) according to methods similar to those described previously (Li et al. 2003). Briefly, twisted bipolar electrodes were constructed from Teflon-coated tungsten wires (50 μm inner core diameter, 75 μm external diameter). Field excitatory postsynaptic potentials (EPSPs) were recorded in the stratum radiatum of the dorsal hippocampus in response to stimulation of the ipsilateral Schaffer collateral–commissural pathway. The recording site was located 3.4 mm posterior to bregma and 2.5 mm lateral to midline, and the stimulating site was located 4.2 mm posterior to bregma and 3.8 mm lateral to midline. In order to record basal synaptic responses, a stimulation electrode was positioned in the stratum oriens, and the far-field inverted EPSP was recorded from the electrode located in stratum radiatum using the same stereotactic coordinates (Leung et al. 2003). The final depths of the electrodes were adjusted to optimize the electrically evoked EPSP and confirmed by postmortem analysis. Experiments commenced after the animals had fully recovered from surgery and were used to being handled (usually about 2 weeks after surgery). After surgery, the rats were housed individually in their home cage between recording sessions with free access to food and water and a 12-h lights on/off cycle. Schaffer collaterals/commissural fibers were stimulated at a frequency of 0.033 Hz and at an intensity evoking half-maximum EPSP amplitude. One hour after baseline recording, LTP was induced using 200 Hz HFS consisting of 10 trains of 20 pulses (intertrain interval of 2 s). Hippocampal electroencephalography (EEG) was monitored between recordings of the evoked EPSPs from the same electrodes, using 25.6 s epochs and a sampling rate of 100 Hz. The power (mV ms) frequency spectrum of the EEG was calculated by fast Fourier transformation with a Hamming window, using the modulus of the amplitude (PowerLab Scope version 4 for Macintosh, ADInstruments Ltd., Oxford, UK). We chose to analyze the 6–8 Hz band because the relative contribution of frequencies within the theta range can be used as an indicant of hippocampal activation (Green and Arduini 1954; Xu et al. 1998).

Recording Apparatus and Novelty Exposure

Experiments were carried out in a well-lit room. The recording compartment consisted of the base of the home cage, including normal bedding and food/water, but the sides were replaced with a translucent Perspex plastic box (27 × 22 × 30 cm) with an open roof. The rats had access to food and water throughout the whole recording session from the same position as in the home cage. All animals were first habituated to the recording procedure over the postsurgery recovery period.

Active exploration of novel objects was achieved by gently introducing objects (elastic ball, glass bottle, cube, toy truck, and dark tissue paper) that were known to attract the continued attention of the rats and increase hippocampal activation as measured by EEG theta activity (Fig. 1). Unless otherwise stated, the animals were exposed to novelty for 25-35 min. Novelty exposure was always begun by placing one small elastic ball very gently near the nose of the

animal. Once the attention of the animal was drawn to the ball, then the ball was placed on the floor out of immediate reach of the animal. If the animal did not move to explore the ball, the attention-drawing procedure was repeated until the animal moved actively to explore the ball. Once the animal stopped exploring the ball for 30 s, another small object was introduced near the animal using the same attention-drawing method. After 4 objects, including the ball, had been explored, clean dark blue tissue paper was inserted gently, totally covering the 4 walls of the recording compartment and the whole floor including the animal and the 4 small objects. The tissue paper and small objects were gently removed from the recording compartment 35 min after starting the novelty exposure protocol. A video camera was used to monitor activity during the whole procedure. Four animals, which explored the novel objects for less than 25 min, were excluded from the analysis. None of the animals displayed behavioral signs of stress such as attempting to escape or behavioral freezing during the novelty procedure.
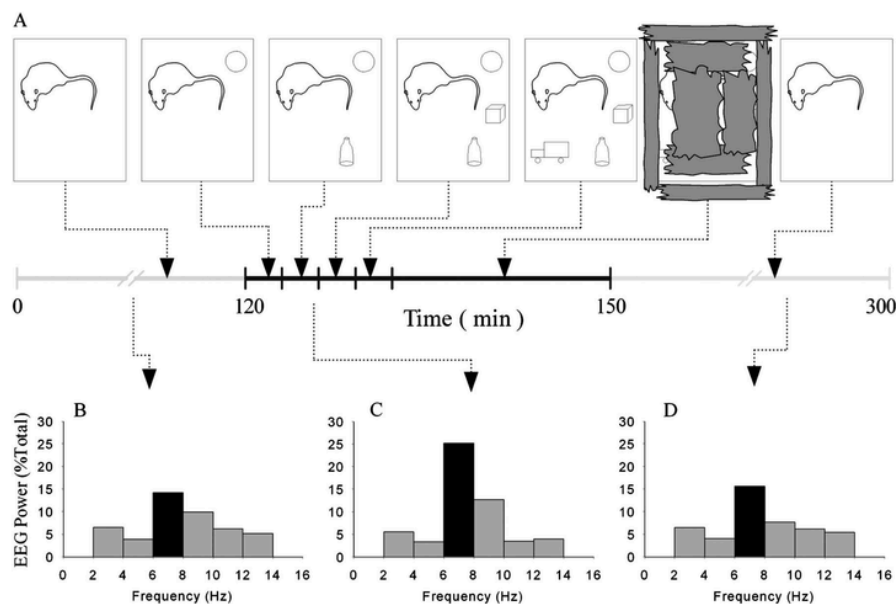
Figure 1.

Figure 1.

Method used to evoke continuous novelty exploration and associated hippocampal EEG activation. (A) Typical novelty exposure sequence. Baseline hippocampal EEG and synaptic field potentials were initially recorded from the chronically implanted rat in the modified home cage for a 120-min period. Then, novel objects were introduced gradually over a period of approximately 25–35 min. Once the animal stopped actively investigating the novel object, a new object was introduced to the animal. In the example illustrated, 4 consecutive

approximately 3-min periods of gently introducing a ball, bottle, cube, and toy truck were followed by a further approximately 18-min period when the rat and objects were enveloped in dark tissue paper. The novel objects were then removed gently and recording continued for a further 150 min. (B–D) Typical examples of the relative power of different EEG frequency bands. In the example shown, the relative power in the theta frequency band of 6–8 Hz increased markedly during the period of exploration of the novel objects (C) compared with the baseline (B) and post-novelty (D) periods. Values are expressed as the % total power in the 25.6 s recording epoch.

Drugs

The group II mGluR antagonist LY341495 (2S,1_S,2_S)-2(9-xanthylmethyl)-2-(2-carboxycyclopropyl) glycine (Tocris, Bristol, UK) was initially dissolved in 0.1 M NaOH. The mGlu5R antagonist 2-methyl-6-(phenylethynyl) pyridine (MPEP, Ascent Scientific, Bristol, UK) was dissolved directly in sterile 0.9% saline. The other mGlu5R antagonist, 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP, Ascent Scientific), was initially dissolved in distilled water. The NMDAR antagonist (R)-3-(2-carboxypiperazin-4-yl) propyl-1-phosphonic acid (CPP, Ascent Scientific) was also initially dissolved in distilled water. The GluN2A subunit–selective NMDAR antagonist (R)-[(S)-1-(4-bromo-phenyl)-ethylamino]-(2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-5-yl)-methyl)-phosphonic acid (NVP-AAM077, Sigma) was prepared in saline. The GluN2B subunit–selective NMDAR antagonist Ro 25-6981 (αR,βS)-α-(4-hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidinepropanol hydrochloride, Sigma, Ireland) was initially dissolved in dimethyl sulfoxide (Sigma). Final drug solutions were made up in sterile 0.9% saline and adjusted to a pH of 7.4–7.8 with HCl before administration. Control vehicle injections consisted of an equivalent volume of normal saline including an equivalent amount of initial solvent (total volume 1.0–1.5 mL/rat). All drugs were administered by i.p injection 30 min post-HFS.

Data Analysis

Both the magnitude of LTP and the power of 6–8 Hz EEG frequency band are expressed as the percentage of baseline during the initial 60-min period, expressed as mean ± standard error of the mean, unless otherwise stated. For statistical analysis, EPSP amplitude and EEG power were grouped into 10-min epochs. One-way analysis of variance (ANOVA) with repeated measures was used to compare the magnitude of LTP over the 4 h post-HFS period within the experimental groups followed by post hoc Bonferroni multiple comparison tests. Standard one-way ANOVA was used to compare the magnitude of LTP between multiple groups followed by post hoc Tukey's tests. Unpaired Student's t-tests were used for 2-group comparisons. A $P < 0.05$ was considered statistically significant.

Results

Novelty Exploration Persistently Reverses LTP

Consistent with previous findings, exploration of a non-aversive novel environment reversed LTP of synaptic transmission in the CA1 area of freely

behaving animals (Xu et al. 1998; Manahan-Vaughan and Braunewell 1999). In control animals, HFS induced a robust LTP that persisted for at least 4 h (159.0 ± 5.9% and 154.2 ± 6.1% pre-HFS baseline EPSP amplitude at 1 and 4 h post-HFS, respectively, n = 8; P < 0.05 compared with pre-HFS baseline; P > 0.05 comparing 1 and 4 h) (Fig. 2A). In marked contrast, exploration of a series of novel objects for 25–35 min, which were introduced 1 h after HFS, almost completely reversed LTP (150.0 ± 7.0% and 112.9 ± 6.2% at 1 and 4 h post-HFS, respectively, P < 0.05, n = 7; P > 0.05 compared with controls at 1 h and P < 0.05 compared with controls at 4 h) (Fig. 2B). Similar to our previous findings, the reversal of LTP was relatively rapid in onset and persisted for the duration of recording. We also confirmed that the same novelty exposure did not significantly affect baseline synaptic transmission in the absence of prior HFS (100.8 ± 1.2% at 4 h after introducing novelty, n = 5, P > 0.05 compared with pre-novelty baseline; P > 0.05 compared with the magnitude of baseline transmission at an equivalent time point in animals that were not exposed to novelty; 100.2 ± 1.8%, n = 5) (Fig. 2A,B).
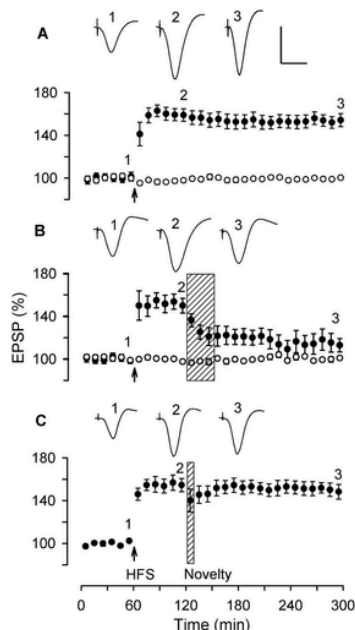
Figure 2.

Figure 2.

Novelty exploration triggers a rapid and persistent reversal of LTP in freely behaving rats. (A) HFS (arrow) induced stable LTP of excitatory transmission at CA3-CA1 apical synapses recorded in the stratum radiatum of the dorsal hippocampus in control rats (closed circles, n = 8). Baseline synaptic

transmission remained stable for the recording period in rats that did not receive HFS (open circles, n = 5). (B) Whereas exploration of novel objects for a period of 25–30 min (novelty, crosshatched bar) did not affect baseline synaptic transmission in the absence of prior HFS (open circles; n = 5), the same duration of novelty exploration, starting 1 h after HFS, rapidly caused a strong persistent reversal of previously established LTP (closed circles, n = 7). (C) In contrast, exploration of novel objects for a briefer period of 5–10 min (crosshatched bar), starting 1 h after HFS, failed to persistently reverse previously established LTP (closed circles, n = 5). Insets show representative traces at the times indicated. Calibration bars: 20 ms, 2 mV.

Next, we determined if briefer periods of exploration, which we previously found to facilitate LTP induction (Li et al. 2003), were sufficient to induce depotentiation. Interestingly, novelty exploration lasting between 5 and 10 min was insufficient to induce a persistent depotentiation (154.5 ± 6.2%, 148.2 ± 6.9% pre-HFS baseline EPSP amplitude at 1 and 4 h post-HFS, respectively, n = 5; $P < 0.05$ compared with pre-HFS baseline; $P > 0.05$ comparing 1 and 4 h) (Fig. 2C).

The strong reversal of LTP by novelty exploration indicates that the process may be widespread across the CA3-CA1 synaptic network. We therefore examined the ability of novelty exploration to reverse LTP also at basal synapses in the CA1 area.

Similar to apical synapses, 25–30 min exploration of novelty strongly and persistently reversed LTP at basal synapses that had been induced 1 h previously by HFS (186.4 ± 7.5% at 1 h and 119.3 ± 5.4% at 4 h post-HFS, n = 6, $P < 0.05$; $P > 0.05$ and $P < 0.05$ compared with 174.7 ± 8.7% and 153.2 ± 7.5% in controls at 1 and 4 h post-HFS, respectively, n = 6) (Fig. 3).

Figure 3.

Switching off LTP: mGlu and NMDA Receptor–Dependent Novelty Exploration–Induced Depotentiation in the Rat Hippocampus
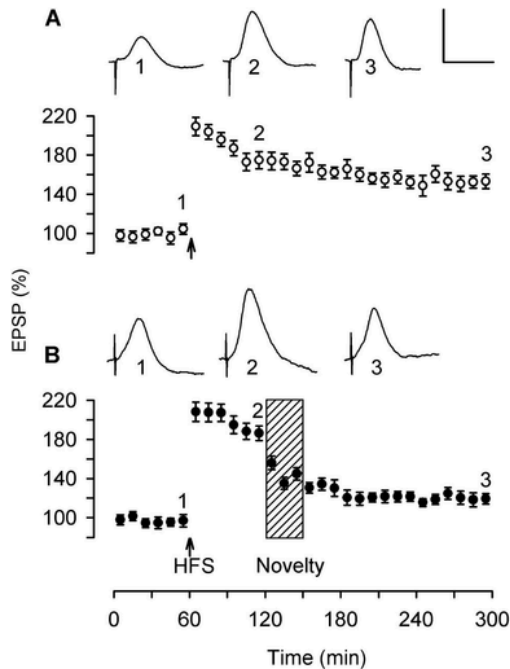
Figure 3.

Novelty exploration also induces a reversal of LTP at basal synapses (A) HFS (arrow) triggered a persistent LTP at CA3-CA1 basal synapses in the stratum oriens of control rats (n = 6). (B) In contrast, LTP was rapidly and persistently reversed by the exploration of novelty for a period of 25–30 min (crosshatched bar) starting 1 h after HFS (n = 6). Insets show representative traces at the times indicated. Calibration bars: 20 ms, 2 mV.

mGluR-Dependence of Novelty Exploration-Induced Depotentiation

We assessed the involvement of group I and group II mGluRs in the reversal of LTP caused by novelty exposure using a combination of the mGlu5R antagonist MPEP (3 mg/kg, i.p.) in conjunction with the group II mGluR antagonist LY341495 (3 mg/kg, i.p.) (Barker et al. 2006). Whereas in animals that were injected with vehicle 30 min post-HFS novelty exploration significantly reversed LTP (155.6 ± 5.8% and 113.7 ± 6.7% at 1 and 4 h post-HFS, respectively, n = 7; $P < 0.05$), co-injection of MPEP and LY341495 prevented the reversal of LTP by novelty exposure (158.2 ± 7.7% and 154.3 ± 5.6% at 1 and 4 h post-HFS, respectively, n = 6; $P > 0.05$ between times; $P < 0.05$ compared with vehicle) (Fig. 4A). Co-injection of this dose combination of MPEP and LY341495 30 min post-HFS did not significantly affect LTP persistence in animals that were not exposed to novelty (161.3 ± 7.9%, 162.3 ± 6.6%, and 152.1 ± 6.9% at 30 min, 1 h, and 4 h

post-HFS, respectively, n = 4; P > 0.05 comparing 30 min with either 1 or 4 h, not shown).
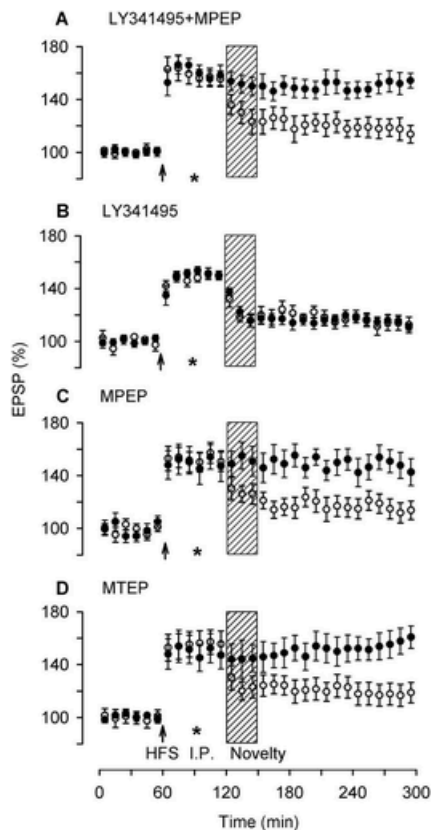
Figure 4.

Figure 4.

mGluR-dependence of novelty-induced depotentiation. (A) Whereas novelty exploration (crosshatched bar) triggered depotentiation in animals that received a vehicle injection i.p. (asterisk, i.p.) 30 min after HFS and 30 min prior to the introduction of novel objects (open circles, n = 7), the same duration of novelty exploration failed to induce depotentiation in animals that were injected with a combination of the group II mGluR antagonist LY341495 (3 mg/kg, i.p.) and the mGlu5R antagonist MPEP (3 mg/kg, i.p.) (closed circles, n = 6). (B) Treatment with the group II mGluR antagonist LY341495 alone (3 mg/kg, i.p., closed circles, n = 5) did not inhibit the reversal of LTP by novelty exploration (vehicle controls, open circles, n = 5). (C) Unlike vehicle-injected animals (open circles, n = 5), novelty exploration failed to reverse LTP in animals that were injected with MPEP alone (closed circles, 3 mg/kg, i.p., n = 6). (D) Administration of another mGlu5R antagonist MTEP (closed circles, 3 mg/kg, i.p., n = 6) also prevented novelty-induced depotentiation, whereas LTP was persistently reversed by novelty exploration in vehicle-injected rats (open circles, n = 7).

Role of mGlu5Rs in Novelty Exploration–Induced Depotentiation

Having found that combined antagonism of group I and group II mGluRs prevented the reversal of LTP by novelty exploration, next we examined the role

of group II mGluRs by testing the effect of LY341495 (3 mg/kg, i.p.) alone. This treatment did not significantly affect ability of novelty exploration to reverse LTP (149.2 ± 2.3% and 110.8 ± 2.6% at 1 and 4 h post-HFS, respectively, n = 5; P > 0.05; P > 0.05 compared with 112.4 ± 5.9% at 4 h, from 150.0 ± 3.3%, in vehicle-injected controls, n = 5) (Fig. 4B). The involvement of mGlu5Rs was assessed using MPEP alone and another mGlu5R subtype-selective antagonist MTEP (Varty et al. 2005). MPEP (3 mg/kg) alone prevented the reversal of LTP induced by novelty exploration (147.1 ± 11.7% and 142.5 ± 10.1% at 1 and 4 h post-HFS, respectively, n = 6; P > 0.05; P < 0.05 compared with 113.7 ± 7.0% at 4 h, from 150.6 ± 7.5%, in vehicle-injected controls, n = 5) (Fig. 4C). Similar to animals that received co-injection of MPEP with LY341495, LTP was not significantly affected by MPEP alone (3 mg/kg) when administered 30 min post-HFS (150.0 ± 8.0, 152.3 ± 7.2, and 142.1 ± 9.3% at 30 min, 1 h, and 4 h post-HFS, respectively, n = 3; P > 0.05, not shown).

Confirmation of the importance of mGlu5Rs was found using MTEP. Thus, whereas in the vehicle-injected control group, novelty exploration reversed LTP (155.6 ± 10.0% and 118.7 ± 7.7% at 1 and 4 h, respectively, n = 7; P < 0.05), in animals that received an MTEP injection (3 mg/kg, i.p.), novelty exploration no longer induced depotentiation (147.1 ± 10.3% and 160.8 ± 8.4% at 1 and 4 h, respectively, n = 6; P > 0.05 comparing times; P < 0.05 compared with vehicle-injected controls) (Fig. 4D). Moreover, LTP persistence was not significantly affected by the i.p. injection of MTEP 30 min post-HFS (149.0 ± 8.5, 146.8 ± 8.3, and 145.8 ± 8.2 at 30 min, 1 h, and 4 h post-HFS, respectively, n = 3; P > 0.05, not shown).

NMDAR-Dependence of Novelty Exploration-Induced LTP Reversal: Role of GluN2B Subunit–Containing NMDARs

To determine whether or not LTP reversal induced by novelty was dependent on NMDARs, we first examined the effect of the general NMDAR antagonist CPP. Injection of CPP (5 mg/kg, i.p.) 30 min prior to novelty exposure and 30 min post-HFS prevented the reversal of LTP (148.1 ± 6.1% and 152.0 ± 4.2% at 1 and 4 h, respectively, n = 6; P > 0.05; P < 0.05 compared with 114.1 ± 8.5% at 4 h, from 153.6 ± 9.2%, in saline vehicle-injected controls, n = 7) (Fig. 5A). This dose of CPP did not significantly affect the persistence of LTP when injected 30 min post-HFS (156.3 ± 8.0%, 157.3 ± 7.2%, and 152.1 ± 9.1% at 30 min, 1 h, and 4 h post-HFS, respectively, n = 3; P > 0.05, not shown).
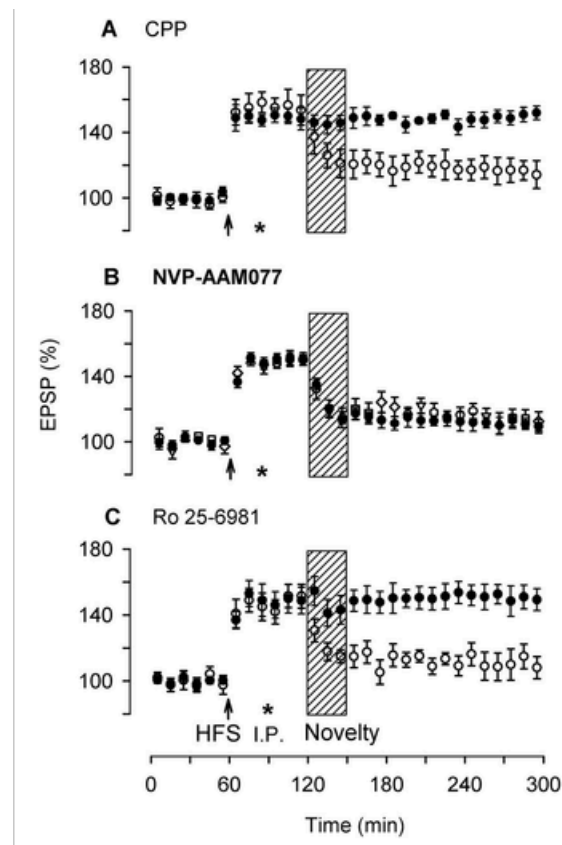
Figure 5.

Figure 5.

NMDAR-dependence of novelty-induced depotentiation. (A) Whereas novelty exploration (crosshatched bar) triggered reversal of LTP in animals that were injected with vehicle i.p. 30 min post-HFS (asterisk, i.p., open circles, n = 7), the same duration of novelty exploration failed to reverse LTP in rats that had been injected previously with the NMDAR antagonist CPP (closed circles, 5 mg/kg, i.p., n = 6). (B) Treatment with an antagonist selective for NMDARs containing GluN2A subunits (1.2 mg/kg, i.p., closed circles, n = 5) did not inhibit the reversal of LTP by novelty exploration (vehicle controls, open circles, n = 5). (C) Novelty exploration triggered depotentiation in rats that received a vehicle injection 30 min post-HFS (open circles, n = 5). In contrast, injection of an antagonist selective for GluN2-containing NMDAR subunits, Ro 25-6981 (closed circles, 6 mg/kg, i.p., n = 6), prevented the novelty exploration-induced reversal of LTP.

GluN2B Subunit–Containing NMDARs Are Required for Novelty Exploration-Induced Depotentiation

Next, we tested the ability of the NMDAR antagonist NVP at a dose (1.2 mg/kg, i.p.) that has been reported to be selective for receptors with GluN2A subunits (Ge et al. 2010). Such treatment failed to significantly affect the reversal of LTP by novelty exploration (150.6 ± 3.9% and 109.4 ± 4.3% at 1 and 4 h post-HFS, respectively, n = 5; $P > 0.05$; $P > 0.05$ compared with 112.4 ± 5.9% at 4 h, from 150.0 ± 3.3%, in vehicle-injected controls, n = 5) (Fig. 5B). The contribution of GluN2B subunit–containing NMDARs in the NMDAR-dependence of LTP reversal

induced by novelty exploration was assessed using a GluN2B subtype–selective antagonist Ro 25-6981. We found that Ro 25-6981 (6 mg/kg, i.p.) injected 30 min prior to novelty exploration prevented the depotentiation. Whereas in the vehicle control group, novelty exploration reversed LTP ($151.4 \pm 7.2\%$ and $108.0 \pm 6.7\%$ at 1 and 4 h, respectively, n = 5; $P < 0.05$), novelty exploration did not significantly affect the magnitude of LTP in the drug-treated group ($148.7 \pm 8.1\%$ and $149.3 \pm 6.6\%$ at 1 and 4 h, respectively, n = 6; $P > 0.05$; $P < 0.05$ compared with $114.1 \pm 8.5\%$ at 4 h, from $153.6 \pm 9.2\%$, in saline vehicle-injected controls) (Fig. 5C) LTP persistence was not significantly affected by Ro 25-6981 when injected 30 min post-HFS ($155.2 \pm 8.1\%$, $156.4 \pm 7.3\%$, and $150.7 \pm 7.8\%$ at 30 min, 1 h, and 4 h, respectively, n = 3; $P > 0.05$, not shown).

Hippocampal EEG Activation during Novelty Exploration

The ability of the drugs to affect hippocampal activation during exploration was investigated by measuring the power of the EEG in the theta band (Green and Arduini 1954; Xu et al. 1998). All the animals explored the novel objects for 25–35 min with no significant difference between experimental groups (Table 1). There was a marked increase in theta power, particularly during the first 20 min of novelty exploration ($P < 0.05$ compared with pre-novelty in controls, n = 4). There was no significant difference between experimental groups during novelty exploration ($P > 0.05$; MTEP, n = 4; CPP, n = 4; Ro 25-6981, n = 6) (Fig. 6).

Table 1

Table 1
Effects of the different drug treatments on the duration of active exploration of the novel objects

Group	LY + MPEP	LY	MPEP	MTEP	CPP	NVP	Ro 25-6981
Vehicle	28.4 ± 1.2 (7)	29.4 ± 1.1 (5)	29.9 ± 1.4 (5)	30.5 ± 1.0 (7)	30.8 ± 0.7 (7)	29.4 ± 1.1 (5)	29.9 ± 0.08 (5)
Drug	29.3 ± 0.9 (6)	29.9 ± 0.06 (5)	30.4 ± 1.4 (6)	29.7 ± 1.7 (6)	29.6 ± 1.7 (6)	30.0 ± 0.08 (5)	28.8 ± 0.9 (6)

Note: Values are the mean ± standard error of the mean minute duration. The number of animals per group is given in parentheses. LY: LY341495; NVP: NVP-AAM077.

Effects of the different drug treatments on the duration of active exploration of the novel objects

Figure 6.

Switching off LTP: mGlu and NMDA Receptor–Dependent Novelty Exploration–Induced Depotentiation in the Rat Hippocampus
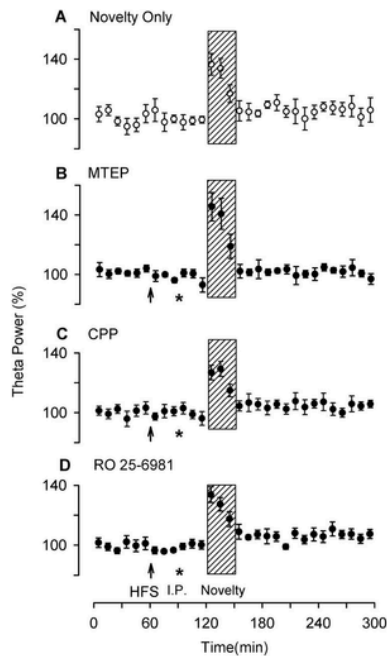
Figure 6.

Increased theta band EEG power in the hippocampus during novelty exploration: lack of significant effect of injection of agents used to block mGluRs or NMDARs. Novelty exploration (crosshatched bar) transiently increased the power (mean ± standard error of the mean % baseline) of the EEG in the 6–8 Hz frequency range in animals that explored the novel objects (A, novelty alone, n = 4). Novelty exploration caused a similar increase in the power of theta activity in rats that had been injected with either MTEP (B, 3 mg/kg, i.p., n = 4; asterisk, i.p.), CPP (C, 5 mg/kg, i.p., n = 4), or Ro 25-6981 (D, 6 mg/kg, i.p., n = 6) 30 min prior to the introduction of novel objects. The times of HFS and injection are indicated by an arrow and asterisk (i.p.), respectively.

Discussion

Prolonged, but not brief, novelty exploration induced a rapid onset and persistent reversal of LTP that appeared to be widespread in the CA1 area of the hippocampus of freely behaving rats. Thus, the magnitude and time course of depotentiation was similar both at apical and basal CA3-CA1 synapses. Further examination of the mechanisms underlying the induction of the depotentiation at apical synapses by novelty found it to be dependent on the activation of both NMDARs and mGluRs. In particular, the requirement for both GluN2B subunit–containing NMDARs and mGlu5Rs was strongly implicated using selective antagonists of these subtypes of glutamate receptors at doses that did not affect novelty-associated activation of the hippocampus, as measured by EEG.

The present data extend our and others' previous findings that exploration of a novel environment triggers depotentiation (Xu et al. 1998; Manahan-Vaughan and Braunewell 1999; Abraham et al. 2002). Unlike previous studies in which the rat was moved to a different environment, here continued exploration of novelty was maintained by gradually introducing novel objects to the animal. The exploration of the changes in the familiar environment was sufficient to trigger increased theta band power in the hippocampal EEG for the duration of the period of novelty exposure. Consistent with our previous findings, the persistent reduction in synaptic strength was limited to potentiated pathways since baseline transmission in a non-potentiated pathway was not reduced by the same novelty exploration. In contrast to the present novelty protocol in which the animals actively explored the novel objects, strong aversive novelty that triggers avoidance or behavioral freezing inhibits the induction of LTP (Foy et al. 1987; Diamond et al. 1994; Hirata et al. 2008). Indeed, allowing animals to actively explore novel objects in a familiar environment for 60 min immediately after inescapable footshock/restraint reverses such stress-evoked inhibition of LTP induction in the CA1 area (Yang et al. 2006). Furthermore, exposure of rats to inescapable stress for 30 min on an elevated platform that triggers behavioral freezing and piloerection, does not reverse LTP at CA3-CA1 synapses (S Li and MJ Rowan, unpublished data). Interestingly, in the present study, active exploration of novel objects for 5–10 min triggered only a transient reversal of LTP. The requirement for more prolonged (~30 min) novelty exploration to induce persistent depotentiation may be due to the ability of brief, but not prolonged, novelty exploration to facilitate LTP induction (Li et al. 2003). Similar opposing processes influence the ability of brief novelty exploration to influence LTP of the population spike in the dentate gyrus (Straube et al. 2003).

In addition to experience-dependent depotentiation being inducible at apical synapses in the CA1 area, we now report a similar rapid and persistent reversal of LTP at basal synapses in this region. Indeed, both LTP- and LFS-induced depotentiation have been reported to be more readily inducible in basal compared with apical synapses, and the cellular mechanisms of LTP induction and consolidation at CA3-CA1 synapses in the stratum oriens and stratum radiatum appear to differ in many ways (Haley et al. 1996; Kramar and Lynch 2003; Leung et al. 2003; Sajikumar et al. 2007). The presence of such widespread persistent reversal of LTP of both basal and apical CA3 inputs to CA1 indicates that novelty exploration has a strong network-wide role in controlling the persistence of previously established synaptic potentiation. Similar network-wide adjustments of synaptic strength are a hallmark of synaptic scaling and homeostasis (Vitureira et al. 2011). Therefore, the possibility that the rapid reversal of LTP is triggered by such homeostatic processes during the prolonged exploration of novelty warrants further investigation.

Because hippocampal EEG activation and the duration of exploration of the novel objects was not different between the different drug conditions, the ability of the antagonists to prevent novelty-induced depotentiation is unlikely to be caused by some nonspecific actions such as reduced arousal or attention. Although it is likely that the antagonists were directly inhibiting depotentiation at CA3-CA1

synapses, we cannot rule out the possibility that the agents were acting via non-hippocampal sites. For example, NMDAR-dependent LTD in the perirhinal cortex has been proposed to mediate object recognition learning (Griffiths et al. 2008), and inhibitors of mGluRs in this region impair long-term object recognition memory (Barker et al. 2006). The finding that behaviorally induced depotentiation in vivo requires mGluRs, and NMDARs lend support to the relevance of in vitro studies of mGluR- and NMDAR-dependent LFS-induced depotentiation. Moreover, in contrast to most reports on LFS-induced LTD in vitro in which there is a dichotomy between NMDAR- and mGluR-dependent forms, both receptor classes are required in vivo for novelty-induced depotentiation, as shown by the present findings, and novelty-facilitated LTD (Popkirov and Manahan-Vaughan 2011). Both receptors have been reported to be necessary for LFS to induce depotentiation, and the involvement of specific subtypes is beginning to be addressed (Fujii et al. 1991; Huang and Hsu 2001). Extensive in vitro studies have elucidated the cellular mechanisms of mGlu5R-dependent LTD (Gladding et al. 2009; Collingridge et al. 2010; Luscher and Huber 2010). Interestingly, mGlu5R has been implicated particularly in mediating the extinction of fear conditioning and the reversal of previously learned spatial learning (Xu et al. 2009). Likewise, much research on NMDAR-dependent LTD may be relevant to the cellular mechanisms of novelty-induced depotentiation (Collingridge et al. 2010). Although the exact role of NMDAR subtypes that contain GluN2B subunits in synaptic plasticity is complex and a matter of ongoing research (Fetterolf and Foster 2011), Ge et al. (2010) proposed a critical and selective requirement for these receptors during the induction of LTD, as opposed to LTP, at CA3-CA1 synapses in freely behaving adult rats. However, a role for these receptors in behaviorally induced persistent synaptic potentiation in dentate granule cells has also been proposed (Valenzuela-Harrington et al. 2007). The present findings extend the role of GluN2B subunit–containing NMDARs to the behavioral induction of depotentiation, providing further evidence that the role of these NMDARs in learning-related plasticity appears to be pleiotropic. Thus, the ability to impair reversal learning of spatial information by pharmacological antagonism or genetic removal of these receptors may not only be due to interference with the induction of LTP (and behaviorally induced potentiation) or LTD (Duffy et al. 2008; von Engelhardt et al. 2008) but also the prevention of depotentiation.

It is hoped that increasing the understanding of the functional significance and mechanisms of synaptic depotentiation in the hippocampus will also help elucidate its role in pathological conditions. For example, hippocampal depotentiation is selectively disrupted in a neurodevelopmental model of schizophrenia (Sanderson et al. 2012), and the trophic factor neuroregulin-1, implicated in schizophrenia susceptibility, induces an activity-dependent depotentiation at CA3-CA1 synapses (Kwon et al. 2005). Targeting mGlu5Rs or GluN2B subunit–containing NMDARs may provide mechanistic insights into potential treatments for such diseases. Overall, the present findings support a potentially important time- and state-dependent physiological role of NMDAR- and mGluR-dependent depotentiation. Furthermore, the engagement of such depotentiation may be involved in the activity-dependent erasure of recently stored information in the hippocampus, showing strong parallels with similar

mechanisms engaged in the amygdala (Kim et al. 2007). A rapid-onset, glutamate receptor-dependent persistent reversal of LTP expression provides strong support for the engagement of extensive synaptic weakening in the enablement of hippocampal storage of new information.

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