Involvement of the Metabotropic Glutamate Receptor mGluR5 in NMDA Receptor-Dependent, Learning-Facilitated Long-Term Depression in CA1 Synapses

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Learning-facilitated synaptic plasticity describes the ability of hippocampal synapses to respond with persistent synaptic plasticity to the coupling of weak afferent stimulation, which is subthreshold for the induction of plasticity, with a spatial learning experience. The metabotropic glutamate receptor subtype 5 (mGluR5) is critically involved in enabling the persistency of multiple forms of hippocampal synaptic plasticity. We compared the effects of pharmacological allosteric antagonism of mGluR5 in learning-facilitated plasticity with plasticity that had been induced solely by patterned afferent stimulation of the Schaffer collateral pathway to the CA1 stratum radiatum of adult freely behaving rats. Intracerebroventricular injection of the selective mGluR5 antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) had no effect on basal synaptic transmission but significantly prevented both long-term depression (LTD) elicited by electrical stimulation and LTD facilitated by novel object-place configuration learning. NMDA receptor antagonism also prevented learning-facilitated LTD. Habituation to the objects was prevented by MPEP application. Whereas reexposure to the object-place configuration (after 7 days) failed to facilitate LTD in control animals, those who had been treated previously with MPEP expressed LTD, suggesting that inhibition of learning contributed to the initial prevention of LTD. These data support a pivotal role for mGluR5 in both hippocampal LTD and the acquisition of object-place configurations.

Keywords: CA1, freely moving, hippocampus, LTD, MPEP, rat, synaptic plasticity

Introduction

Synaptic plasticity has been subject to intensive study over the past 40 years that has led to considerable and detailed insights into the underlying mechanisms (Bliss and Collingridge 1993; Malenka and Bear 2004). Memory formation is generally accepted to rely on synaptic plasticity (Bear 1996; Martin et al. 2000), yet it can be problematic to quantify the interdependence of the two by simultaneous assessment: physiological plasticity mostly remains indiscernible to current electrophysiological recording approaches, and artificially induced plasticity alone is no surrogate for mammalian learning. One approach to unify the behavioral and the electrophysiological aspects of learning is through the study of learning-facilitated plasticity in freely behaving rats (Manahan-Vaughan and Braunewell 1999; Kemp and Manahan-Vaughan 2004, 2007). Here, subthreshold afferent stimulation, which is not adequate for the induction of persistent synaptic plasticity, is coupled with a spatial learning experience to produce durable changes in synaptic responses. This is inducible at many hippocampal synapses, though not uniformly so (Kemp and Manahan-Vaughan 2008b). At CA1 synapses, exploration of minor features of a spatial context facilitates the expression of long-term depression (LTD) (lasting days) after afferent stimulation that normally yields only short-term depression (STD) (Manahan-Vaughan and Braunewell 1999; Kemp and Manahan-Vaughan 2004). Exploration of spatial arrangements of large landmarks does not facilitate LTD in CA1 but does so effectively in the dentate gyrus (DG) (Kemp and Manahan-Vaughan 2008b). At either of these synapses, simply changing the environment suffices to facilitate long-term potentiation (LTP) (Davis et al. 2004; Kemp and Manahan-Vaughan 2004, 2007, 2008b), although exploration of novel space has also been reported to inhibit LTP (Xu et al. 1998). Learning facilitation is tightly regulated by neuromodulators acting on, for example, beta-adrenoceptors (Strahle, Korz, Balschun, and Frey 2003; Kemp and Manahan-Vaughan 2008a; Lemon et al. 2009), 5-HT4 receptors (Kemp and Manahan-Vaughan 2005), or dopamine D1/D5 receptors (Li et al. 2003; Lemon and Manahan-Vaughan 2006). Emotional state also strongly influences the direction and expression of synaptic plasticity (Diamond et al. 2005). Recent data suggest that learning-facilitated plasticity is mechanistically different from plasticity that is induced solely by patterned electrical stimulation of afferent fibers. At CA1 synapses, for example, learning-facilitated LTD depends on the activation of beta-adrenoceptors, while electrically-induced LTD does not (Kemp and Manahan-Vaughan 2008a; Lemon et al. 2009). This suggests that learning-facilitated plasticity should be subject to greater scrutiny. To date, little is known, for instance, about its regulation by the glutamate receptors that are so intrinsically required for multiple forms of hippocampal synaptic plasticity and hippocampus-dependent learning (Balschun and Wetzel 2002; Naie and Manahan-Vaughan 2004, 2005; Manahan-Vaughan and Braunewell 2005; Balschun et al. 2006; Altimbilek and Manahan-Vaughan 2007, 2009; Bikbaev et al. 2008).

Whereas LTP requires the activation of NMDA receptors at both CA1 and DG synapses of freely behaving adult rats (Morris et al. 1986; Manahan-Vaughan et al. 1998; Fox et al. 2006), LTD in vivo depends on the activation of NMDA receptors at CA1 (Thiels et al. 1996; Manahan-Vaughan 1997) but not DG synapses (Wang et al. 1997; Pöschel and Manahan-Vaughan 2007). The involvement of metabotropic glutamate receptors (mGluRs) in persistent (>24 h) synaptic plasticity that is induced solely by means of patterned electrical afferent stimulation (e.g., 1 Hz, 100 Hz) is well documented. Group I mGluRs, that couple positively to phospholipase C, appear critically important for both LTP and LTD (Wibsch et al. 1998; Balschun et al. 1999; Balschun and Wetzel 2002; Naie and Manahan-Vaughan 2004, 2005; Manahan-Vaughan and Braunewell 2005; Bikbaev et al. 2008), whereas group II and...
group III mGluRs, that couple negatively to adenyl cyclase, preferentially regulate LTD (Manahan-Vaughan 1998; Huang et al. 1999; Pöschel et al. 2005; Altimbilek and Manahan-Vaughan 2007, 2009) or depotentiation (Hölischer et al. 1997; Kulla et al. 1999). The role of mGluRs in learning-facilitated plasticity has yet to be explored. Both mGluR1 and mGluR5 regulate synaptic excitability and are important for synaptic plasticity (Mannaioni et al. 2001). mGluR5 is of particular interest, given the high significance of mGluR5 for multiple forms of synaptic plasticity at a variety of hippocampal synapses in vivo (Balschun and Wetzel 2002; Naie and Manahan-Vaughan 2004; Manahan-Vaughan and Braunewell 2005; Welsby et al. 2006; Naie et al. 2007; Neyman and Manahan-Vaughan 2008). Therefore, we investigated whether learning-facilitated plasticity at the CA1 synapses of freely behaving adult rats requires mGluR5.

Materials and Methods

Surgery
Male Wistar rats (Charles River) underwent stereotactic surgery under general anesthesia at postnatal weeks 7–8, as described previously (Manahan-Vaughan 1997). A guide cannula (anterior-posterior [AP]: -0.5; medial-to-lateral [ML]: 1.6) was implanted into the right lateral cerebral ventricle to enable intracerebroventricular injections. Implantation of a bipolar stimulation recording electrode in the Schaffer collateral pathway (AP: -3.1; ML: 3.1) and a recording electrode in stratum radiatum of the CA1 region (AP: -2.8; ML: 1.8) of the right hippocampus was conducted according to established procedures (Manahan-Vaughan and Braunewell 1999). Online test-pulse recordings during surgery aided the depth adjustment of the electrodes, which was later verified by postmortem histology. After surgery, rats recovered for 7–10 days in individual cages with unrestricted food and water supply and were then used for in vivo recordings. Animals were transferred to the experiment room on the day before recordings to acclimatize. Recordings were carried out in 40-cm (length) × 40-cm (width) × 50-cm (height) chambers wherein rats could move freely with unlimited access to food and water.

Measurement of Evoked Potentials
Field excitatory postsynaptic potentials (fEPSPs) were recorded in the CA1 region by delivering a biphasic pulse with a half-wave duration of 0.2 ms in the Schaffer collaterals. An input-output curve was recorded before every experiment (maximal stimulation 900 μA) to determine the maximal obtainable slope value. Recordings of basal synaptic transmission (baseline) were carried out with a stimulation intensity that elicited a slope that was 40% of the maximal value. For each recording 5 pulses, 40 s apart, were averaged. The first 30 min of recordings (6 time points) served as baseline, and the data subsequently obtained were expressed as the mean percentage ± the standard error of the mean of this average baseline value. Then, drug or vehicle was administered and another 30 min of test-pulse stimulation followed. After that, in plasticity experiments, low-frequency stimulation (LFS) was applied and the stability of basal synaptic transmission was then followed for 4 h. On the next day, recordings were made for 1 h. Intracranial electroencephalogram was monitored throughout experiments.

Induction of Synaptic Depression
To ensure stability of recordings, all animals were first tested in a baseline experiment over the same time period as subsequent experiments. LTD was induced by 900 pulses of LFS at 1 Hz with an intensity that yielded an fEPSP slope that comprised 70% of the maximum obtained in the input-output analysis. Subthreshold LFS at 600 pulses (sub-LFS) was applied to induce STD that lasts approximately 60 min. Afferent stimulation of 1 Hz given as 300 pulses (300-pulse LFS) was applied to elicit a weak STD that lasts for approximately 15 min.

The terms “electrically induced” plasticity and “learning-facilitated” plasticity were used to distinguish between synaptic plasticity induced exclusively by electrical stimulation and plasticity that is facilitated by the combination of novel spatial exploration with mild electrical stimulation (that would normally not induce long-lasting plasticity). For analysis of differences between electrophysiological groups, a 2-way analysis of variance (ANOVA) with repeated measures was applied. To assess statistical differences in the subsequent synaptic depression, the fEPSPs from the period after stimulation until the end of the experiments were compared. The level of significance was set at P < 0.05.

Novel Spatial Exploration
To observe the effect of learning on synaptic plasticity, we employed a protocol first described by Manahan-Vaughan and Braunewell (1999) and used a 39 × 39-cm gray hole board that could be inserted into the recording chamber. In all related experiments, the hole board was introduced at the beginning of LFS and removed after 10 min. The hole board contained 4 holes (5.5 cm in diameter and 5 cm deep), equidistant from one another: one in each corner. A small object of unique appearance and size was placed in each hole for the animal to explore. Prior to the second experiment, the animals were exposed to objects that they had never seen before. Reexposure (second exposure) comprises the presentation of the same objects in the same hole board holes. In certain cases, a third exposure took place—here, the now familiar objects were presented in different hole board holes (reconfiguration). Roughly 7 days interleaved each of these 3 exposures. Animals were excluded from analysis if they expressed significant stress (e.g., freezing) or apathy during the exploration.

Habituation
During 15 min of exposure to the object-place configuration, 2 measures of learning behavior were assessed: the number of times the animals dipped their noses into the hole board holes was counted (dipping) and the number of times the animals reared onto their hind limbs (rearing). These measures were assessed when the animals explored the object-place configuration for the first time, during reexposure to this environment roughly 7 days after first exposure, and during a third and final exposure to the same environment a further 7 days after the reexposure. Effects were statistically assessed with Student’s t-test. The level of significance was set at P < 0.05.

Drug Treatment
The negative allosteric mGluR5 modulator 2-methyl-6-(phenylethynyl) pyridine (MPEP; Biozol) was dissolved in 5 μl of 0.9% NaCl to a dose of 1.8 μg. The competitive NMDA antagonist D-(-)-2-amino-5-phosphono-pentanoic acid (D-AP5) was dissolved in 5 μl of 0.9% NaCl and applied in a dose of 19.7 μg. Drug, or vehicle, was injected continuously into the right ventricle over a period of 5 min via a Hamilton syringe. The Hamilton syringe was connected by means of a flexible polyurethane tube to an injection cannula that was inserted into the permanently implanted cannula. Antagonist or vehicle injection was carried out 30 min prior to stimulation to enable diffusion from the lateral cerebral ventricle to the hippocampus to occur (Manahan-Vaughan et al. 1998).

Results
Pharmacological Antagonism of mGluR5 Has No Effect on Basal Synaptic Transmission in the CA1 Region of Freely Behaving Adult Rats
In previous studies, we reported that the selective mGluR5 antagonist, MPEP, has no effect on basal synaptic transmission in the DG of freely moving rats when injected intracerebrally in the amount of 1.8 μg (Naie and Manahan-Vaughan 2004). When the same amount was injected into the lateral cerebral ventricle, no effect on basal synaptic transmission in CA1 synapses was seen over the 24-h monitoring period (Fig. 1; n = 6; ANOVA: F1,549 = 1.0992, P = 0.29516).
Electrically Induced LTD and STD Are Modulated by mGluR5

LFS at 1 Hz, given 900 times, elicited LTD that persisted for over 24 h in vehicle-injected animals (Fig. 2). In controls \((n = 9)\), the fEPSP was initially reduced to \(56.59 \pm 8.49\%\) of baseline values \((t = 5 \text{ min})\). On the following day, LTD was still present (mean fEPSP value: \(68.90 \pm 3.54\%\) at \(t = 24 \text{ h}\); Fig. 2). In the presence of MPEP, LTD was significantly impaired: LFS induced following injection. Vertical scale bar corresponds to 3 mV, and horizontal bar corresponds to 3 ms. Analog traces show the field potentials preinjection and 5 min and 24 h following injection. Vertical scale bar corresponds to 3 mV, and horizontal bar corresponds to 3 ms.

To investigate whether antagonism of mGluR5 influences STD induced by subthreshold LFS (sub-LFS, 1 Hz, 600 pulses), we injected MPEP (1.8 \(\mu\)g) 30 min before stimulation (Fig. 3). Control animals that received sub-LFS \((n = 5)\) expressed STD that persisted for approximately 90 min. Treatment with MPEP significantly reduced STD, with just a small and transient depression appearing immediately after sub-LFS \((n = 5; \text{ANOVA: } P < 0.0001)\).

Learning-Facilitated LTD Is Dependent on Activation of mGluR5

Figure 4 provides a summary of the layout of the learning facilitation experiments. We reported previously that induction of LTD is facilitated by exploration of novel object-place configurations during application of a subthreshold LFS (Manahan-Vaughan and Braunewell 1999; Kemp and Manahan-Vaughan 2004). Here, we examined the effects of mGluR5 antagonism on this phenomenon.

Fifteen animals were given sub-LFS (1 Hz, 600 pulses) to elicited STD—this corresponds to the experimental phase “1” described in the schema in Figure 4. These data are represented in “1 control + test” in Figure 5 \((n = 8 \text{ control } + n = 7 \text{ test}); \text{subthreshold LFS (sub-LFS, 1 Hz, 600 pulses)}\) when given alone induced an STD that returned to baseline levels after approximately 1 h in both control and test groups (Fig. 5). Eight days after the STD assessment, 8 of these animals were treated with vehicle prior to phase “2” of the experimental paradigm (control group) and 7 were treated with MPEP (test group). When the control animals were allowed to explore a novel object-place configuration during the application of sub-LFS, LTD was expressed that lasted for at least 24 h. The average fEPSP slope was 66.88 \(\pm 5.48\%\) \((n = 9)\) 24 h after stimulation (Fig. 5; ANOVA compared to sub-LFS alone: \(F_{1,506} = 342.55, P < 0.0001\)). Treatment with MPEP (1.8 \(\mu\)g) significantly prevented learning-facilitated LTD in test group (Fig. 5; \(n = 7\); ANOVA: \(F_{1,322} = 302.55, P < 0.0001\)) compared with vehicle-treated controls. Effects became evident 15 min after LTD induced by subthreshold LFS.
Figure 4. Schematic summary of the experimental design for learning-facilitated synaptic plasticity. Both "test" and "control" animals first received LFS that is subthreshold for the induction of persistent LTD in the absence of an object-place configuration (1) and in the next 3 experiments in the presence of an object-place configuration (2-4). Whereas control animals received vehicle, test animals received MPEP prior to the first exposure to the object-place configuration (2). Labels above the hole board diagrams describe the object-place configuration, and labels below indicate which icv treatment was administered 30 min before stimulation. Gray arrows indicate the time intervals between experiments in days. Group names and experiment numbers are referenced in Figures 5 and 6 accordingly.

Figure 5. Learning-facilitated plasticity is blocked in the presence of an antagonist of mGluR5. STD that lasts approximately 60 min is induced by sub-LFS (1Hz, 600 pulses) (1 control + 1 test; n = 15; see Fig. 4 for experimental schema). Coupling sub-LFS with the exploration of a novel object-place configuration (2 control) facilitates the expression of LTD that lasts for over 24 h (n = 8). Pharmacological antagonism of mGluR5 using MPEP (1.8 μg; 2 test; n = 7) completely prevents learning-facilitated LTD. Analog traces show the field potentials preinjection, 5 min, 4 h, and 24 h following sub-LFS. Vertical scale bar corresponds to 3 mV, and horizontal bar corresponds to 3 ms.

Figure 6. Rearrangement of object-place configuration in controls and second exposure in test animals facilitates LTD. Roughly 1 week after attempting to induce learning-facilitated LTD in vehicle- or MPEP-treated animals, treatment with sub-LFS (1Hz, 600 pulses) was repeated in the presence of the now familiar object-place configuration (3 control and 3 test; see Fig. 4 for experimental schema). No drug treatment was given. Under these conditions, animals that were previously treated with vehicle did not express LTD (3 control; n = 9), whereas animals that had been treated with MPEP (1.8 μg) before novel exposure to the object-place configuration now expressed robust LTD (3 test; n = 7). On next exposure, control animals responded to a rearranged object-place configuration with LTD facilitation (4 control; n = 8). Test group animals were presented with the same configuration once more and did not express LTD (4 test; n = 7). Analog traces show the field potentials preinjection, 5 min, 4 h, and 24 h following sub-LFS. Vertical scale bar corresponds to 3 mV, and horizontal bar corresponds to 3 ms.
Hole Board Exploration Facilitates Even Very Weak STD
The question remained whether the inhibition of LTD facilitation by MPEP is indeed related to impaired learning or whether the effect of MPEP relates merely to the consequence of an upstream block of LTD mechanisms. To clarify this, we first stimulated a group of animals \((n = 6)\) with very weak LFS (300 pulses). This elicited a very small STD (average fEPSP of first 3 values after stimulation was 90% of baseline; Fig. 8) that resembled the remnant response after 600-pulse LFS of MPEP-treated animals (see black squares in Fig. 2). This small STD was facilitated into a small but persistent LTD when stimulation was coupled to 10 min of novel object-place exploration (Fig. 8; \(n = 6\); ANOVA: \(F_{1,230} = 116.74, P < 0.0001, n = 6\). Thus, an inhibition of synaptic depression alone would not explain the block of learning-associated facilitation seen previously.

Learning-Facilitated LTD Is Blocked by an NMDA Antagonist
LTD in CA1 in vivo can be blocked by D-AP5, a competitive NMDA antagonist (Manahan-Vaughan 1997). To find out whether NMDA receptors are also involved in our model of learning-facilitated LTD, we injected D-AP5 (19.7 \(\mu g\)) prior to stimulation and exposure to a novel object-place configuration. While in control experiments induction of STD coupled with novel object-place exploration facilitated robust LTD, D-AP5 blocked this depression completely (Fig. 9; \(n = 6\); ANOVA: \(F_{1,215} = 213.55, P < 0.0001\)). This indicates that the NMDA pathway, crucial to electrically-induced LTD expression in CA1 (Manahan-Vaughan 1997), is also involved in learning-facilitated LTD.

Pharmacological Antagonism of mGluR5 Prevents Habituation to a Spatial Environment
To examine whether the inhibition of learning-facilitated LTD, by antagonism of mGluR5, was associated with any effects on learning, we compared habituation of vehicle- \((n = 6)\) and MPEP-treated animals \((n = 6)\) upon second and third exposures to the now familiar object-place configuration (roughly 1 and 2 weeks after the first exposure, respectively). Previously, we had shown that a marked habituation effect is evident in controls (Manahan-Vaughan and Brauneewell 1999). Here, a similar effect was seen, but in MPEP-treated animals, no habituation was evident when animals explored the object-place configuration for a second time (Fig. 10). In control animals, a significantly reduced dipping and rearing behavior was evident when the first exposure was compared with the second exposure.
habituation behavior in both the control and the MPEP-treated groups. The same object-place configuration, roughly 1 week after the second exposure, revealed no significant differences in behavior. Control animals also showed habituation to this environment (Fig. 6).

MPEP animals learned the "novel" environment and exhibited a significantly reduced dipping and rearing behavior (Fig. 10). Thus, MPEP prevented learning of the new object-place configuration, suggesting that MPEP did not directly affect exploratory behavior. However, no significant difference in behavior was observed when the third exposure was compared with vehicle-treated controls (Fig. 10), indicating that the dependency of synaptic plasticity on mGluR5 in the DG may relate more strongly to its regulation of intracellular calcium release or to other functions such as suppression of the calcium-activated potassium current (Mannaioni et al. 2001) and increases in neuronal excitability that occur independently of activation of phospholipase C and inositol trisphosphate (Ireland and Abraham 2002; Rae and Irving 2004). Antagonism of mGluR5 significantly impairs LTD in hippocampal slices in vitro (Harney et al. 2006; Neyman and Manahan-Vaughan 2008). Effects are possibly due to an inhibition of mGluR5-mediated NMDA receptor currents (Harney et al. 2006) and subsequent alteration of intracellular calcium levels (Harney et al. 2006; Naie et al. 2007). In the CA1 region of postnatal (11–35 days old) rats, a dissociation of NMDA receptor-dependent and mGluR-dependent forms of LTD is evident (Nicol et al. 1998), whereas in adult rats, the NMDA receptor and group I mGluR contribution to LTD appear to be intertwined (Manahan-Vaughan 2007).

MPEP-treated animals did not express a significantly different dipping and rearing behavior when first exposure was compared with vehicle-treated controls (Fig. 10), suggesting that MPEP did not directly affect exploratory behavior. However, no significant difference in behavior was evident in the MPEP-treated group when first and second exposures were compared (P = 0.4381 for dips and P = 0.653 for rears). Thus, MPEP prevented learning of the new object-place configuration, and MPEP-treated animals behaved, upon reexposure to this environment, as if they had never experienced it before. Upon the third exposure, to the same object-place configuration, a significantly reduced dipping and rearing behavior was evident (P < 0.001 and P < 0.001 for both dips and rears), suggesting that during the second exposure, MPEP animals learned the "novel" environment and exhibited habituation upon reexposure (in this case their third exposure). Control animals also showed habituation to this environment (Fig. 6).

Discussion

The results of this study indicate that mGluR5 is critically required for both electrically induced and learning-facilitated LTD in the CA1 region of freely behaving adult rats. The impairment of learning-facilitated LTD was associated with an inhibition of habituation to the novel object-place configuration, suggesting that mGluR5 is required for both the hippocampal LTD and the acquisition of novel spatial information.

MPEP is predominantly postsynaptically localized, couples positively to phospholipase C via Gq, and mediates phosphoinositide hydrolysis resulting in calcium release from intracellular stores (Valenti et al. 2002). In the CA1 region, both LTP and LTD critically depend on activation of NMDA receptors (Morris et al. 1986; Dudek and Bear 1992; Manahan-Vaughan 1997). This may explain the strong regulation of synaptic plasticity by mGluR5 in this structure. In other hippocampal structures, such as the DG, LTP can be induced by activation of NMDA receptors and/or voltage-gated calcium channels (Manahan-Vaughan et al. 1998) and LTD does not require NMDA receptor activation (Pöschel and Manahan-Vaughan 2007). Thus, the dependency of synaptic plasticity on mGluR in the DG may relate more strongly to its regulation of intracellular calcium release or to other functions such as suppression of the calcium-activated potassium current (Mannaioni et al. 2001) and increases in neuronal excitability that occur independently of activation of phospholipase C and inositol trisphosphate (Ireland and Abraham 2002; Rae and Irving 2004). Antagonism of mGluR5 significantly impairs LTD in hippocampal slices in vitro (Harney et al. 2006; Neyman and Manahan-Vaughan 2008). Effects are possibly due to an inhibition of mGluR5-mediated NMDA receptor currents (Harney et al. 2006) and subsequent alteration of intracellular calcium levels (Harney et al. 2006; Naie et al. 2007). In the CA1 region of postnatal (11–35 days old) rats, a dissociation of NMDA receptor-dependent and mGluR-dependent forms of LTD is evident (Nicol et al. 1998), whereas in adult rats, the NMDA receptor and group I mGluR contribution to LTD appear to be intertwined (Manahan-Vaughan 2007).

MPEP plays a critical role in the acquisition of spatial memory by rodents. Transgenic animals that lack mGluR5 show deficient learning in the water maze (Lu et al. 1997), whereas animals that received repeated treatment with an mGluR5 antagonist show marked impairments in spatial learning in either an 8-arm radial maze (Naie and Manahan-Vaughan 2004; Manahan-Vaughan and Braunewell 2005; Bikbaev et al. 2008) or a spatial alternation task (Balschun and Wetzel 2002). Conversely, positive allosteric modulation of mGluR5 enhances both LTP and LTD, as well as spatial learning (Balschun et al. 2006; Ayala et al. 2009). It is quite striking that the level of expression of mGluR5 in the rodent hippocampus relates to spatial learning ability (Manahan-Vaughan and Braunewell 2005): the higher the expression, the better the learning ability. In fragile X syndrome, a disorder that is associated with marked mental retardation, exaggerated mGluR5 signaling is implicated (Do¨len and Bear 2008), whereas mGluR5-mediated synaptic plasticity is absent in fragile X mental retardation protein knockout mice (Wilson and Cox 2007). The picture emerges that normal functioning of mGluR5 may be pivotal for normal learning and normal synaptic plasticity (Do¨len and Bear 2008; Conn et al. 2009).
In recent years, LTD has emerged as a candidate mechanism for synaptic information storage that likely partners LTP in the generation of spatial memories in response to sensory experience (Bear 1996; Braunewell and Manahan-Vaughan 2001; Kemp and Manahan-Vaughan 2007). Although LTD was posited for many years as comprising the mechanism underlying spatial learning (Morris et al. 2003), it has also been reported that preventing LTD impairs spatial learning (Nakao et al. 2002; Etkin et al. 2006). Furthermore, the combination of weak low-frequency afferent stimulation with the acquisition of information about a novel spatial context facilitates the expression of very persistent LTD (Kemp and Manahan-Vaughan 2007). Learning facilitation of LTD thus involves the coupling of afferent stimulation to the hippocampus that is subthreshold for the induction of persistent synaptic plasticity, with a novel spatial learning event. We would like to emphasize, however, that the term "learning-facilitated" plasticity was very carefully chosen. We do not claim that we are "inducing" LTD, that is, what would amount to learning-induced plasticity. Our model examines the relationship between spatial learning and associated changes in synaptic plasticity that are long lasting and could suggest a correlation with learning, but this is not the same as a clear induction of synaptic plasticity by a learning event.

Here, we allowed the animals to explore a novel hole board that contained small partially concealed objects in the hole board holes. Exploration in the form of head-dipping into the holes and rearing was significantly different when the first exposure was compared with the second exposure, indicating that the animals had habituated to and learned about the environment. The first novel exposure to this novel spatial context resulted in a facilitation of STD into LTD. Reexposure to the same environment approximately 1 week after the first exposure did not result in LTD when the animals received weak afferent stimulation of the Schaffer collaterals to CA1. This suggests a direct association between the novel learning event and the facilitation of LTD, in line with previous reports from our lab (Manahan-Vaughan and Braunewell 1999; Kemp and Manahan-Vaughan 2004, 2007, 2008b; Lemon and Manahan-Vaughan 2006). The facilitation of LTD derives not from the novelty of the objects themselves but from the novelty of the objects' relative position in space (Kemp and Manahan-Vaughan 2004). This suggests that the facilitation of LTD occurs as a result of a "spatial" learning event. In accordance with this postulate, we have shown in the past that the prevention of spatial learning by application of either antagonists of dopamine D1/D5 receptors (Lemon and Manahan-Vaughan 2006) or beta-adrenergic receptors (Kemp and Manahan-Vaughan 2008b; Lemon et al. 2009) is associated with a failure to facilitate LTD during the novel exploration event.

In the present study, we report for the first time that both electrically induced and learning-facilitated LTD, in the hippocampal CA1 region in vivo, are prevented by antagonism of mGluR5. This suggests that mGluR5 may play a very particular role in enabling forms of synaptic plasticity that involve a depression of synaptic strength. Our study reveals that concentrations of MPEP that prevent persistent LTP and spatial learning (Naie and Manahan-Vaughan 2004; Manahan-Vaughan and Braunewell 2005; Bikbaev et al. 2008) also prevent persistent LTD elicited by LFS in vivo. We additionally show that antagonism of mGluR5 prevented habituation to the novel spatial environment (during the first object-place exposure) and prevented learning-facilitated LTD. When the animals were reexposed to the same environment roughly 1 week after MPEP-treatment, facilitation of LTD occurred that was accompanied by exploration of the novel environment that was akin to the first exposure, that is, animals behaved as if they had never seen the environment before. A subsequent (third) exposure to the same object-place configuration failed to facilitate LTD and revealed that the animals had now habituated to the environment. This suggests that antagonism of mGluR5 prevented learning of the spatial environment and provides an interesting link between this phenomenon and the facilitation of LTD. Given the significance of protein synthesis for learning (Rozenzweig 1996), it is tempting to speculate that the regulation by mGluR5 of spatial learning and LTD reported here are related to the ability of mGluR5 to trigger dendritic protein synthesis (Huber et al. 2001; Naie et al. 2007) and the protein synthesis dependency of LTD in the CA1 region in vivo (Manahan-Vaughan et al. 2000).

It was striking that the synaptic depression that was evident immediately after LFS was given during hole board exploration during mGluR5 antagonism was much larger than the synaptic depression we observed when LFS was given in the presence of MPEP under control (nonlearning) conditions. This may reflect the increased activity of hippocampal inputs from, for example, the entorhinal cortex due to the sensory processing of the novel spatial environment and/or increased activity of neuro-modulatory systems such as the above-mentioned noradrenergic or dopaminergic input to the hippocampus. This would presumably result in a higher level of hippocampal excitability and a reduced threshold for the induction of synaptic plasticity (Tsanaou and Manahan-Vaughan 2008, 2009; Lemon et al. 2009).

The effects of MPEP on learning-facilitated LTD were not state dependent. Thus, treatment with the mGluR5 antagonist prior to the first novel exposure and to the reexposure to the hole board was equally effective in preventing learning-facilitated plasticity. One can also exclude that the failure to induce learning-facilitated plasticity in the presence of the mGluR5 antagonist was due to the fact that the magnitude of STD elicited in the presence of the antagonist was too small to engage in facilitation by spatial learning. Electrical induction of a very small STD—equivalent to that which occurred when LFS was given in the presence of MPEP—when coupled with novel spatial context learning, still resulted in learning-facilitated plasticity. Interestingly, learning-facilitated plasticity was also prevented when an NMDA receptor antagonist was applied. This is consistent with previous observations that NMDA antagonists prevent both synaptic plasticity and spatial learning (Morris et al. 1986; Manahan-Vaughan 1997). This also suggests that activation of NMDA receptors upstream of or coincident with the activation of mGluR5 is an intrinsic part of learning-facilitated plasticity. NMDA receptor currents are facilitated by activation of mGluR5, and high concentrations of MPEP can suppress this regulation (Mannaioni et al. 2001). We observed an impairment of the early phase of LTD by MPEP. In previous studies, however, we showed that the same amount of MPEP injected intracerebrally in the current study (1.8 μg) impairs the late phases of persistent LTP in vivo but has no effect on the early NMDA receptor-dependent component of LTP (Manahan-Vaughan and Braunewell 2005; Naie and Manahan-Vaughan 2005). The reduction in the early component of LTD in the presence of the mGluR5 antagonist may reflect an impairment.
of the NMDA receptor contribution to LTD induction (i.e., calcium currents elicited by prolonged weak activation of NMDA receptors during LFS might be more vulnerable to inhibition of mGluR5 than calcium currents elicited during tetanization to induce LTP) or it may reflect effects on dendritic protein synthesis (Huber et al. 2001). In the present study, in contrast to effects in the presence of MPEP where a small depression was seen, no synaptic depression occurred when AP5 was given, suggesting that NMDA receptor activation is required for the early phase of LTD/STD. Therefore, one can speculate that the effects of NMDA receptor and GluR5 antagonism on learning-facilitated plasticity reflect distinct components of the molecular cascade underlying this process.

Although antagonism of mGluR5 prevented both electrically-induced LTD (and STD) and learning-facilitated LTD, these forms of LTD may not be mediated by the same intracellular phenomena. At CA1 synapses, learning-facilitated LTD requires the activation of beta-adrenergoreceptors, whereas electrically induced LTD does not (Kemp and Manahan-Vaughan 2008a; Lemon et al. 2009). Beta-adrenergoreceptors are positively linked to adenylyl cyclase via Gs proteins and can thus lead to intracellular elevations of protein kinase A (PKA). PKA is considered an important element for many forms of hippocampal synaptic plasticity including LTD (Nguyen and Woo 2003). Elevation of PKA levels derives not only from activation of beta-adrenergoreceptors but also from a variety of other G-coupled receptors such as the dopamine D1/D5 receptors and cholinergic muscarinic receptors. The differences in beta-adrenergoreceptor modulation of electrically induced LTD and learning-facilitated LTD may thus suggest that they are distinct phenomena. However, these differences may also relate to the relative degree of activation of AMPA-coupled neurotransmitter receptors as a result of strong afferent activation via potent electrical stimulation or weaker afferent activation when weak electrical stimulation is coupled with a learning event.

Conclusions
Our data support that mGluR5 is critically required for both electrically induced and learning-facilitated LTD, as well as the learning of object-place configurations. These data not only support a pivotal role for mGluR5 in hippocampal plasticity and hippocampus-dependent learning but also offer further support to the possibility that LTD is involved in spatial learning.

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