Hippocampal long-term depression mediates spatial reversal learning in the Morris water maze

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1. Introduction

It is widely held that the cellular mechanism underlying learning and memory in the brain is activity-dependent synaptic plasticity, e.g., N-methyl-D-aspartate receptor (NMDAR)-dependent long-term potentiation (LTP) and long-term depression (LTD) (Bliss and Collingridge, 1993; Collingridge et al., 2010; Malenka and Nicoll, 1999). Behavioral experience may generate endogenous synaptic plasticity to dynamically modulate the induction threshold for subsequent hippocampal LTP and LTD (Kemp and Manahan-Vaughan, 2004; Manahan-Vaughan and Braunewell, 1999). However, most previous experiments have focused on the potential role of LTP in learning and memory (Lynch, 2004; Malenka and Nicoll, 1999; Martin et al., 2000). Correlations between behavioral experience and LTD modulation have not been extensively investigated. Early speculation that LTD may serve as a reversal mechanism for LTP, or a forgetting mechanism, assumed that LTP encodes memories (Stanton, 1996; Tsumoto, 1993). However, recent reports also indicate that LTD plays important roles in processing new information. For example, hippocampal LTD is facilitated by exposure to a novel environment with novel objects or novel configurations of objects (Kemp and Manahan-Vaughan, 2004; Manahan-Vaughan and Braunewell, 1999) and we have recently found that induction of hippocampal CA1 LTD promotes the consolidation of spatial learning in freely moving rats (Ge et al., 2010).

The exact mechanisms underlying the involvement of hippocampal LTD in learning and memory are still not clear, partially due
to the difficulty of inducing LTD with classical low frequency stimulation (LFS) protocols in adult animals (Staubli and Scafidi, 1997; Wong et al., 2007; Xu et al., 1998b). To better understand the role of hippocampal LTD in learning and memory, it is necessary to use a behavioral model in which experience may decrease the induction threshold of hippocampal LTD so that the subsequent classical LFS induces LTD. Several groups have recently investigated the potential role of hippocampal LTD in spatial reversal learning in the Morris water maze. Reversal learning occurs following initial spatial learning by changing the location of hidden platform in water maze. A recent report shows that exogenous d-serine the potential role of hippocampal LTD in spatial reversal learning in classical LFS induces LTD. Several groups have recently investigated the role of hippocampal LTD in learning and memory, it is necessary House Center) were pair-housed in plastic cages in a temperature-controlled (21 °C) colony room on a 12:12 h light/dark cycle. Food and water were available ad libitum. All experiment protocols were approved by the University of British Columbia Animal Care Committee and Chongqing Medical University Animal House Center. All experiment protocols were approved by the University of British Columbia Animal Care Committee and Chongqing Medical University Animal House Center. All efforts were made to minimize the number of animals used.

2. Materials and methods

2.1. Subjects

Adult male Sprague–Dawley rats (300–350 g; obtained from University of British Columbia Animal Care Centre and Chongming Medical University Animal House Center) were pair-housed in plastic cages in a temperature-controlled (21 °C) colony room on a 12:12 h light/dark cycle. Food and water were available ad libitum. All experiment protocols were approved by the University of British Columbia Animal Care Committee and Chongqing Medical University Animal House Center. All efforts were made to minimize the number of animals used.

2.2. Drugs and treatment procedures

The selective GluN2B antagonist Ro25-6981 was purchased from Sigma–Aldrich Inc. (St Louis, MO, USA). GluA2(869YKECYGNVYG77) in the carboxyl-terminal region of the AMPA GluA2 subunit and scrambled (869VYKYGGYNE877) peptide were synthesized by GL Biochem Ltd. (Shanghai, China). To render the peptide membrane permeable and allow it to be applied systemically, we fused the peptide to the cell membrane transduction domain of the HIV-1 protein to make a Tat-GluA23Y peptide (YGRKKRRQRRR-869YKECYGNVYG77) or scrambled Tat-GluA23Y peptide (YGRKKRRQRRR-869VYKYGGYNE877). Ro25-6981 (6.0 mg/kg for i.p. and 0.5 mmol/µl per side for intrahippocampal infusion) and the peptides (3.0 mmol/µl, i.p. and 100 pmol/µl per side for intrahippocampal infusion) were dissolved in 0.5% sterile saline and applied before the first trial of each reversal training day or low frequency stimulation (LFS) in electrophysiological recording.

2.3. Electrophysiological recording

Field EPSPs from the CA1 region of the hippocampus were recorded using techniques described previously (Dong et al., 2006b; Wong et al., 2007). Rats were anesthetized with urethane (1.5 g/kg, i.p.; Sigma, St. Louis, MO) and placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). Rectal temperature was maintained at 36.5 ± 0.5 °C over the course of the experiment with a homeothermic blanket (Harvard Apparatus, Holliston, MA). The scalp was opened, and trephine holes were drilled for the recording (AP ~ 3.5 mm, ML 2.8 mm) and stimulating electrodes (AP ~4.2 mm, ML 3.5 mm). A third hole was drilled anterior and lateral to bregma for the recording electrode ground wire. The recording electrode (130 μm Teflon-coated platinum iridium wire; A-M Systems Inc, Castle Acre, WA) was lowered into the CA1 region, and the stimulating electrodes (tungsten bipolar-stimulating electrode; FHC Inc., Bowdoin, ME) were placed in the Schaffer collaterals of the dorsal hippocampus through the holes. Stimulation pulses (0.12 ms) were generated with an AD analog digital converter (BNC-2090; National Instruments, Austin, TX) and a digital stimulus isolation unit (Gettys Instruments, San Diego, CA). Field EPSPs were recorded with a differential AC preamplifier (Model P55, Grass Technologies, Astro-Med, Inc.) and LabVIEW data acquisition system (National Instruments, Austin, TX). Test EPSPs were evoked at a frequency of 0.033 Hz and at a stimulus intensity adjusted to around 50% of the maximal response size. Low frequency stimulation protocol (LFS) consisted of 900 pulses at 1 Hz. EPSP amplitude was expressed as mean ± SEM % of the baseline EPSP slope recorded over at least a 40-min baseline period, and those in the last 5 min recordings were averaged in one animal and then across animals to give baseline response for the group. In the control group (untreated, Fig. 1A), rats were subjected to swimming in the pool with the hidden platform absent for 4 trials per day with 10 min inter-trial intervals for 4 consecutive days. Swim time was matched to the platform search time in the Morris water maze learning group (MWM, Fig. 1A). Immediately after the last trial of spatial learning, rats were anaesthetized with urethane for fEPSP recording. Following surgery and baseline recording (~ 1.5 h), LFS was delivered. When necessary, Ro25-6981 or Tat-GluA23Y peptide was injected 1 h before delivery of LFS (Fig. 1C).

2.4. Bilateral hippocampal microinjection

Rats were chronically implanted with cannulae above dorsal hippocampus as previously described (Dong et al., 2006a; Ge et al., 2010). Briefly, under sodium pentobarbital anesthesia (60 mg/kg, i.p.), rats were implanted with two 22 Ga stainless steel guide cannulae (10 mm; Plastics One Inc., Roanoke, VA) above the dorsal hippocampus (3.8 mm posterior to bregma, 2.5 mm lateral to the midline and 2.5 mm below the surface of the dura) that were fixed to the skull with four jeweler’s screws and dental cement. Sterile dummy cannula (30 Ga stainless steel rod, 10 mm; Plastics One Inc.) was inserted into guide cannula to avoid bacterial infection and cerebral spinal fluid leakage through the cannula. All rats were allowed to recover for 7 days before behavioral experiments.

On the day before experiments, the animals were placed in the experiment room and given a sham intrahippocampal injection to get acclimatized to the injection procedure. Dummy cannulas were removed and the rats were placed into a Plexiglas injection box (25 × 45 × 25 cm, same as homecage) with 30 Ga injection cannulas in their guide cannulae. Injection cannulae (11 mm, Plastics One Inc., Roanoke, VA) were connected to a microsyringe pump (Harvard Apparatus) by PE-50 tubing, except for IS012m beyond the tip of the guide cannula.

Drugs were injected with 10–µl Hamilton syringes and a microsyringe pump at 0.5 µl/min for 2 min. After injection, the injection cannulas were left in place for an additional minute to allow the diffusion of the drug away from the cannula tip. The rats were then removed from the injection box, their dummy cannulae replaced, and then they were placed back in their home cages. Cannulae placement was verified by histological examination of the brain after methylene blue injection (1 µl per side), and only data obtained from rats with correctly inserted cannulas were included in statistical analysis.

2.5. Water maze tests

Spatial learning and memory was performed in the Morris water maze using procedures similar to those described previously (Ge et al., 2010; Wong et al., 2007). The Morris water maze consisted of a circular fiberglass pool (200 cm diameter) filled with water (25 ± 1 °C) made opaque with black non-toxic paint. The pool was surrounded by light blue curtains, and three distal visual cues were fixed to the curtains. Four floor light sources of equal power provided uniform illumination to the pool and testing room. A CCD camera suspended above the pool center recorded the swim paths of the animals and video output was digitized by an EthoVision tracking system (Noldus, Leesburg, VA). The water maze tests include 3 periods: initial spatial training, spatial reversal training and probe test.

Initial spatial training: Twenty-four hours before spatial training, animals were allowed to adapt to the maze for a 60 s free swim. Rats were then required to swim to find a hidden platform (15 cm × 15 cm, located at NW) submerged 1 cm below the water surface. Rats were placed into the water facing the pool wall at four starting positions [N, S, E, and W]. Animals were then trained in the initial spatial learning task for 4 trials per day with 10 min inter-trial intervals for 4 consecutive days. During each trial, rats were allowed to swim until they found the hidden platform where they remained for 20 s before being returned to a holding cage. Rats that failed to find the hidden platform in 60 s were guided to the platform where they remained for 20 s.

Spatial reversal training: After the initial spatial learning task, a reversal learning protocol was conducted with some rats. During reversal learning, the hidden platform was moved to the opposite quadrant (e.g., from NW to SE). Reversal learning entailed 3 additional days with 4 trials per day, similar to initial training. Rats exposed

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to acute stress were trained with a modified reversal protocol that could be completed in a single session. This protocol was used to avoid confounding the results by exposing the rats to acute stress more than once. Probe test: Twenty-four hours after the final reversal training trial, rats were returned to the pool from a novel drop point with the hidden platform absent for 60 s and their swim path was recorded.

2.6. Elevated-platform stress

Behavioral stress was induced similarly to that described previously by placing rats on an elevated Plexiglas platform (1 m tall, 21 × 21 cm) in a brightly lit room for 30 min immediately before the first reversal training trial (Wong et al., 2007).

2.7. Statistical analysis

For electrophysiological data, LTD was assessed using paired t-test compared with baseline. Between-groups comparisons were conducted by one-way ANOVA followed by Fisher's test. Data from behavioral assessments were analyzed by using two-way ANOVA followed by LSD test. Significance level was set at p < 0.05.

3. Results

3.1. Spatial learning enabled LFS to induce LTD in hippocampal CA1 area in vivo

Previous studies have shown that LTD is difficult to induce in adult rats (Staubli and Scafidi, 1997; Wong et al., 2007; Xu et al., 1998b). Consistent with these results, we found that a typical LFS protocol (1 Hz for 15 min) failed to induce hippocampal CA1 LTD in control rats that were exposed to free swimming trials in the Morris water maze (untrained: 99.6 ± 1.2%, p = 0.340 vs. baseline, Fig. 1A). Since previous reports show that hippocampal-dependent novelty acquisition (Manahan-Vaughan and Braune new, 1999) or passive spatial perception (Kemp and Manahan-Vaughan, 2011) facilitates hippocampal LTD, we next wanted to determine if spatial learning in the Morris water maze facilitates hippocampal CA1 LTD induction. Here, we found the same LFS protocol induced a reliable hippocampal CA1 LTD in rats subjected to 4-day spatial learning in the Morris water maze (MWM: 83.9 ± 2.6%, p < 0.001 vs. baseline, p < 0.001 vs. untrained, Fig. 1A). Notably, water maze training did not affect basal synaptic transmission because the input-output curve remained unchanged in both untrained control and the Morris water maze training groups (Fig. 1B).

It is well known that hippocampal CA1 LTD induced by LFS is dependent on NMDA receptors (Collingridge et al., 2010; Dudek and Bear, 1992; Malenka and Bear, 2004; Mulkey and Malenka, 1992). Further experiments showed that GluN2B-containing NMDA receptors are involved in hippocampal LTD induction (Fox et al., 2006; Ge et al., 2010; Izumi et al., 2006; Liu et al., 2004; Wang et al., 2006; Wong et al., 2007; Woo et al., 2005). To determine whether the hippocampal CA1 LTD induced by LFS after spatial learning depends on GluN2B-containing NMDA receptors, we used the selective GluN2B antagonist Ro25-6981 1 h before the LFS delivery. Similar to results of previous in vivo reports (Fox et al., 2006; Ge et al., 2010; Wang et al., 2006; Wong et al., 2007), systemic application of Ro25-6981 (6 mg/kg, i.p.) prevented hippocampal LTD (Fig. 1C, D). In contrast, a typical LFS protocol (900 pulses at 1 Hz) failed to induce hippocampal CA1 LTD in rats subjected to 4-day spatial learning (untrained: 99.6 ± 1.2%, p = 0.340 vs. saline, Fig. 1C, D).

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While hippocampal CA1 LTD induction depends on NMDA receptors, the expression of LTD involves facilitation of clathrin-dependent endocytosis of postsynaptic AMPA receptors, through an AMPA receptor GluA2 subunit-dependent mechanism (Lüscher et al., 1999; Man et al., 2000). Systemic injection of Tat-GluA23Y peptide, which interferes with the endocytosis of AMPA receptors, also blocks LTD induced by a paired-burst protocol (Fox et al., 2007; Ge et al., 2010). We sought to examine the effect of Tat-GluA23Y peptide on hippocampal CA1 LTD induced by LFS after spatial learning. Administration of the Tat-GluA23Y peptide (Tat-GluA23Y; 3 μmol/kg, 100.1 ± 8.8, p = 0.436 vs. baseline, p < 0.001 vs. scr-Tat-GluA23Y, Fig. 1C, D) completely blocked hippocampal CA1 LTD expression, compared to scrambled peptide (scr-Tat-GluA23Y; 3 μmol/kg, 86.1 ± 1.0%, p < 0.001 vs. baseline, Fig. 1C, D).

Taken together, these results indicate that the LTD induced by LFS after spatial learning in the hippocampal CA1 area requires activation of GluN2B-containing NMDA receptors and the endocytosis of AMPA receptors.

3.2. Systemic administration of Ro25-6981 or Tat-GluA23Y peptide impairs spatial reversal learning

Since hippocampal CA1 LTD induced by LFS after spatial learning is blocked by the selective GluN2B antagonist Ro25-6981, we next wanted to determine the role of GluN2B-containing NMDA receptors in new learning (e.g., spatial reversal learning). After the 4-day spatial learning task, rats were required to perform a spatial reversal learning task, in which the platform location was moved to the opposite quadrant. Rats injected with Ro25-6981 (6.0 mg/kg, i.p.) 1 h before the first trial on each reversal learning day showed a dramatic deficit in performance relative to saline controls (p = 0.001 for the first reversal training day (day 5); p = 0.004 for the second reversal training day (day 6) and p = 0.626 for the third reversal training day (day 7), Fig. 2A). Detailed analysis of escape latencies on each trial revealed that the rats were significantly slower on trial 2 of day 5 and trial 1, 2, and 4 of day 6 (day 5: p = 0.104 for the first trial, p = 0.025 for the second trial, p = 0.073 for the third trial, p = 0.085 for the fourth trial; day 6: p = 0.015 for the first trial, p = 0.003 for the second trial, p = 0.086 for the third trial, p = 0.028 for the fourth trial; day 7: p = 0.063 for the first trial, p = 0.460 for the second trial, p = 0.079 for the third trial, p = 0.482 for the fourth trial, Fig. 2B). However, Ro25-6981 treatment had no effect on memory retrieval that was tested 24 h after the last reversal training trial compared with saline treatment (MWM location: p = 0.147; rMWM location: p = 0.157, Ro25-6981 vs. saline, Fig. 2C). These results indicate that the induction of hippocampal LTD is required for spatial reversal learning in water maze.

Since debate exists regarding whether activation of GluN2B-containing receptors is required for LTD induction (Morishita et al., 2007), we also used a specific inhibitor of LTD expression, the Tat-GluA23Y peptide, which is structurally and mechanistically distinct from GluN2B receptor antagonists, to test the effect of LTD on reversal learning. Systemic application of Tat-GluA23Y peptide (3.0 μmol/kg, i.p.) 1 h before the first trial on each reversal learning day, significantly impaired reversal learning on the second reversal learning day relative to scrambled peptide (p = 0.163 for day 5 and p = 0.044 for day 6 and p = 0.525 for day 7, Fig. 2D). Further analysis of escape latency on each trial revealed that trials 1 and 2 on day 6 were the most deficient trials (day 5: all trials not significantly different; day 6: p = 0.043 for the first trial, p = 0.024 for the second trial, p = 0.222 for the third trial, p = 0.215 for the fourth trial; day 7: all trials not significantly different, Fig. 2E). However, Tat-GluA23Y peptide treatment had no effect on memory retrieval that was tested 24 h after the last reversal training trial (MWM location: p = 0.310; rMWM location: p = 0.379, Tat-GluA23Y vs. scr-Tat-GluA23Y, Fig. 2F). Thus, these data complement the results found with Ro25-6981, indicating that the expression of LTD may play an important role in reversal learning.

3.3. Intrahippocampal infusion of Ro25-6981 or Tat-GluA23Y peptide impairs spatial reversal learning

Systemic application of GluN2B antagonist Ro25-6981 or Tat-GluA23Y peptide may affect the induction and expression of LTD in brain regions other than the hippocampus. To confirm that hippocampal LTD in particular is required for spatial reversal learning, we next examined reversal learning performance of rats that received bilateral intrahippocampal infusion of Ro25-6981 or Tat-GluA23Y peptide 30 min before spatial reversal training. Rats that received bilateral intrahippocampal infusion of Ro25-6981 (0.5 nmol/μl per side) 30 min before the first trial on each reversal learning day showed a dramatic deficiency in performance relative to saline controls (p = 0.030 for the first reversal training day (day 5); p = 0.001 for the second reversal training day (day 6) and p = 0.229 for the third reversal training day (day 7), Fig. 3A). Detailed analysis of escape latencies on each trial revealed that the rats were significantly slower on trial 2 of day 5, trial 2 and 3 of day 6 and trial 1 of day 7 (day 5: p = 0.480 for the first trial, p = 0.025 for the second trial, p = 0.208 for the third trial, p = 0.204 for the fourth trial; day 6: p = 0.075 for the first trial, p = 0.003 for the second trial, p = 0.001 for the third trial, p = 0.269 for the fourth trial; day 7: p = 0.032 for the first trial, p = 0.395 for the second trial, p = 0.254 for the third trial, p = 0.233 for the fourth trial, Fig. 3B). However, Ro25-6981 treatment had no effect on memory retrieval that was tested 24 h after the last reversal training trial compared with saline treatment (MWM location: p = 0.146; rMWM location: p = 0.456, Ro25-6981 vs. saline, Fig. 3C). These results indicate that the induction of hippocampal LTD is required for spatial reversal learning in water maze.

Similarly, bilateral intrahippocampal infusion of Tat-GluA23Y peptide (100 pmol/μl per side) 30 min before the first trial on each reversal learning day, significantly impaired reversal learning task on the first and second reversal learning day relative to scrambled peptide (p = 0.018 for day 5 and p < 0.001 for day 6 and p = 0.476 for day 7, Fig. 3D). Further analysis of escape latency on each trial revealed that trials 4 of day 5 and all trials of day 6 were the most deficient trials (day 5: p = 0.467 for the first trial, p = 0.055 for the second trial, p = 0.077 for the third trial, p = 0.026 for the fourth trial; day 6: p = 0.040 for the first trial, p = 0.003 for the second trial, p = 0.009 for the third trial, p = 0.028 for the fourth trial; day 7: all trials not significantly different, Fig. 3E). However, Tat-GluA23Y peptide treatment had no effect on memory retrieval that was tested 24 h after the last reversal training trial (MWM location: p = 0.359; rMWM location: p = 0.130, Tat-GluA23Y vs. scr-Tat-GluA23Y, Fig. 3F). Together, these data confirm that hippocampal LTD is required for spatial reversal learning in Morris water maze.

3.4. Elevated-platform stress enhanced spatial reversal learning

The results in Figs. 2 and 3 suggest that performance of reversal learning requires hippocampal LTD. Abundant evidence has shown that acute stress facilitates LTD induction (Kim et al., 1996; Wong et al., 2007; Xu et al., 1997). Therefore, it is possible that acute elevated-platform stress may enhance spatial reversal learning. To test this hypothesis, we first needed to determine whether hippocampal LTD facilitated by acute stress depends on GluN2B activation and AMPA receptor endocytosis. Consistent with previous reports, we here found that acute elevated-platform stress for 30 min dramatically facilitated hippocampal CA1 LTD induction in vehicle control group (saline: 81.6 ± 1.8%, p < 0.001 vs. baseline, Fig. 4A);
and the LTD was completely blocked by GluN2B antagonist Ro25-6981 treatment (Ro25-6981: 99.7 ± 2.0%, p = 0.281 vs. baseline, Fig. 4A) as has been shown previously (Wang et al., 2006; Wong et al., 2007). In addition, further experiments showed that hippocampal LTD facilitated by stress is also blocked by the AMPA receptor endocytosis inhibitor Tat-GluA2Y peptide, but not scrambled Tat-GluA2Y peptide (scr-Tat-GluA2Y: 81.2 ± 2.1%, p < 0.001 vs. baseline; Tat-GluA2Y: 98.7 ± 1.5%, p = 0.439 vs. baseline, Fig. 4A) as has been shown previously (Fox et al., 2007). These results suggest that hippocampal CA1 LTD facilitated by both spatial learning and acute stress may share the same mechanisms of induction and expression. Thus, using acute stress to facilitate hippocampal LTD is a feasible means to test the possibility that the facilitation of hippocampal LTD may enhance spatial reversal learning.

In these behavioral experiments, 8 reversal training trials were performed during a period of 30 min immediately following exposure to acute elevated-platform stress. Although stress exposure did not affect the average escape latency of all 8 reversal learning trials on day 5 (p = 0.288, Fig. 4B), detailed analysis of escape latency for each trial revealed that elevated-platform stress
enhanced reversal learning in the first 2 trials relative to non-stress control group (p = 0.032 for the first trial and p = 0.028 for the second trial, Fig. 4C). Furthermore, rats exposed to stress showed better memory for the new reversal target location during a 60 s probe trial 24 h following reversal training day, compared to non-stress control group (p = 0.441 for control group, p = 0.016 for stress group, MWM vs. rMWM location, Fig. 4D). These results provide further evidence that hippocampal LTD plays a critical role in spatial reversal learning.

4. Discussion

The main findings of this study are that spatial learning facilitates the induction of hippocampal LTD, which depends on activation of GluN2B-containing NMDA receptors and the endocytosis of AMPA receptors. Subsequent spatial reversal learning is disrupted by blocking the induction and expression of hippocampal CA1 LTD with two mechanistically and structurally distinct inhibitors (Ro25-6981 and Tat-GluA23Y peptide); and is enhanced by
facilitating hippocampal CA1 LTD with acute elevated-platform stress. Taken together, these findings provide evidence for the role of hippocampal LTD in mediating spatial reversal learning in the water maze.

4.1. Normal spatial learning facilitates hippocampal CA1 LTD

It is well known that the hippocampal LTD is difficult to induce by classical LFS in adult rats (Staubli and Scafidi, 1997; Wong et al., 2007; Xu et al., 1998b). However, the present experiments show that hippocampal CA1 LTD can be induced by LFS after spatial learning in the water maze (Fig. 1A). On the other hand, we found that spatial learning has no effect on basal synaptic transmission as the input–output relationship remains unchanged after spatial learning. Thus, under our experimental conditions, the spatial learning facilitates the induction of hippocampal LTD, rather than depotentiation. It is interesting to note that previous reports have shown associative learning causes a detectable increase in synaptic transmission in certain pathways of the hippocampus using multiple-electrode recordings (Gruart et al., 2006; Whitlock et al., 2006). Discrepancies between these previous studies and the present work remains to be determined, but may be at least in part accounted for by different behavioral and electrophysiological recording protocols used in these studies.

NMDA receptors are critical for many forms of synaptic plasticity, including the induction of hippocampal CA1 homosynaptic LTD (Dudek and Bear, 1992; Malenka and Bear, 2004; Mulkey and Malenka, 1992). The potential relationship between the subtypes of NMDA receptors activated and the induction of either LTP or LTD has been the subject of intense investigation. Although the requirement of GluN2B-containing receptors for LTD induction in vitro is still under debate (Bartlett et al., 2007; Hendricson et al., 2002; Izumi et al., 2006; Liu et al., 2004; Morishita et al., 2007; Wool et al., 2005; Yang et al., 2005), in vivo studies from different groups have consistently shown that hippocampal LTD induction in the CA1 region requires activation of GluN2B-containing NMDA receptors (Fox et al., 2006; Ge et al., 2010; Wang et al., 2006; Wool et al., 2007). Consistent with these results, we found hippocampal CA1 LTD induced after spatial learning in the water maze depends on the activation of GluN2B-containing NMDA receptors, since the LTD was completely blocked by the selective GluN2B antagonist Ro25-6981. Besides the contribution of GluN2B receptors to hippocampal LTD induction, AMPA receptors are also critical for hippocampal LTD, since the expression of LTD requires endocytosis of postsynaptic AMPA receptors (Bredt and Nicoll, 2003; Collingridge et al., 2004; Malinow and Malenka, 2002), through an AMPA receptor GluA2 subunit-dependent mechanism (Luscher et al., 1999; Man et al., 2000). Previous reports show that the synthetic peptide Tat-GluA2(2-3Y) blocks LTD by interfering with facilitated endocytosis of AMPA receptors, the last step of LTD expression, without affecting any upstream signaling steps (Ahmadian et al., 2004; Brebner et al., 2005; Fox et al., 2007; Ge et al., 2010). Consistent with these reports, we found that hippocampal LTD induced after spatial learning was completely blocked...
by systemic administration of Tat-GluA23Y peptide. Therefore, these results demonstrate the potential utility of the two structurally and mechanistically distinct LTD inhibitors in probing the role of hippocampal LTD in spatial reversal learning.

4.2. Blocking hippocampal LTD impairs spatial reversal learning

Recent evidence shows that systemic injection of Ro25-6981 impairs spatial reversal learning but not initial spatial learning in mice using a water maze task (Duffy et al., 2008). Consistent with these data, we found that rats injected with Ro25-6981 took longer to find a new platform location during the reversal learning phase of a water maze task (Figs. 2 and 3), suggesting that spatial reversal learning was impaired after the blockade of GluN2B-containing NMDA receptors. Furthermore, we found that Tat-GluA23Y peptide administration also impaired spatial reversal learning (Figs. 2 and 3), suggesting that spatial reversal learning was impaired after prevention of the endocytosis of AMPA receptors. Combined with the electrophysiological results that the induction and expression of hippocampal LTD are blocked by Ro25-6981 and Tat-GluA23Y peptide respectively, these results indicate that hippocampal CA1 LTD is necessary for spatial reversal learning in the water maze.

4.3. Facilitating hippocampal LTD enhances spatial reversal learning

In the present study, we found that acute elevated-platform stress enhanced spatial reversal learning and memory (Fig. 4). These results are supported by a recent report that moderate stress enhances late reversal learning in a mouse touchscreen-based choice task (Graybeal et al., 2011). While it is tempting to speculate that acute stress enhances reversal learning by facilitating LTD (Kim et al., 1996; Wong et al., 2007; Xu et al., 1997), acute stress also impairs hippocampal CA1 LTD (Cazakoff and Howland, 2010; Diamond et al., 1994; Kim et al., 2005). Thus, one possibility is that acute stress (and therefore LTD) impairs retrieval of previously encoded information and subsequently enables more rapid learning about the new platform location during reversal learning (Nicholls et al., 2008). This hypothesis is supported by previous reports showing that acute stress disrupts the retrieval of hippocampus-dependent spatial memory (Diamond et al., 1996; Wong et al., 2007; Woodson et al., 2003) although it does not significantly affect the ability of the animals to learn spatial tasks (Diamond et al., 1999; Kim et al., 2005). An alternative explanation is that LTD facilitated by stress may enhance behavioral flexibility by enabling the nervous system to acquire new information and/or reduce the interference of new learning from previously acquired information via resetting the critical hippocampal circuitry (Collingridge et al., 2010; Duffy et al., 2008). This idea is supported by electrophysiological observations that LTP is reversed or depotentiated by exploration of a novel environment (Kemp and Manahan-Vaughan, 2004; Xu et al., 1998a). Therefore, LTD may function to increase signal-to-noise ratio between acquired memory and new learning, and subsequently prevent previous traces from interfering with new information encoding when the demands of a task change.

5. Conclusions

In summary, spatial reversal learning in the water maze is impaired by blocking the induction or expression of hippocampal CA1 LTD with two mechanistically and structurally distinct inhibitors; and it is potentiated by facilitation of hippocampal LTD with acute elevated-platform stress. These results provide evidence that hippocampal LTD may be both necessary and sufficient to mediate behavioral flexibility and play a critical role in new information processing.

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