

# mGluR5 Has a Critical Role in Inhibitory Learning

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The mechanisms that contribute to the extinction of previously acquired memories are not well understood. These processes, often referred to as inhibitory learning, are thought to be parallel learning mechanisms that require a reacquisition of new information and suppression of previously acquired experiences in order to adapt to novel situations. Using newly generated metabotropic glutamate receptor 5 (mGluR5) knock-out mice, we investigated the role of mGluR5 in the acquisition and reversal of an associative conditioned task and a spatial reference task. We found that acquisition of fear conditioning is partially impaired in mice lacking mGluR5. More markedly, we found that extinction of both contextual and auditory fear was completely abolished in mGluR5 knock-out mice. In the Morris Water Maze test (MWM), mGluR5 knock-out mice exhibited mild deficits in the rate of acquisition of the regular water maze task, but again had significant deficits in the reversal task, despite overall spatial memory being intact. Together, these results demonstrate that mGluR5 is critical to the function of neural circuits that are required for inhibitory learning mechanisms, and suggest that targeting metabotropic receptors may be useful in treating psychiatric disorders in which aversive memories are inappropriately retained.

## Introduction

Metabotropic glutamate receptors (mGluRs) modulate neural activity via their linkage to various intracellular cascades (Nakanishi, 1992). The eight individual mammalian mGluRs can be subdivided into three groups based on their sequence homologies and physiological activities. mGluR5 belongs to the Group I mGluRs and is coupled to inositol phosphate/Ca<sup>2+</sup> signal transduction pathway (Abe et al., 1992). mGluR5 has been demonstrated to have important roles in several forms of synaptic plasticity (Lu et al., 1997; Jia et al., 1998; Huber et al., 2000; Bikbaev et al., 2008) and learning behaviors (Lu et al., 1997; Chiamulera et al., 2001; Balschun and Wetzell, 2002), and has been suggested as a potential therapeutic target in several neurological disorders (Brody et al., 2004; Slassi et al., 2005; Marino and Conn, 2006; Dölen et al., 2007). mGluR5 is expressed throughout the CNS including in the hippocampus and lateral nucleus of the amygdala (Abe et al., 1992), which are both structures central to learning and memory mechanisms. Previous studies have demonstrated a role for mGluR5 in two very different forms of learning associated with the hippocampus and the amygdala. Systemic injection (Schulz et al., 2001) or local amygdala perfusion (Rodrigues et al., 2002) of mGluR5 antagonists can disrupt the acquisition of the fear response, and contextual fear conditioning is impaired in mice deficient for mGluR5 (Lu et al., 1997). Similarly mGluR5 knock-out mice show deficits in the acquisition of hippocampal dependent learning (Lu et al., 1997). Thus, while there

is significant evidence that mGluR5 is involved in the acquisition of new memories and synaptic plasticity mechanisms, the role of mGluR5 in one important aspect of learning is unknown: the reversal or extinction of a previously acquired task, an important adaptive process that corrects for an altered environmental or situation (Bouton, 1993). These learning mechanisms are important to retasking, and are particularly relevant to anxiety disorders in humans such as phobias and post-traumatic stress disorder (Barad, 2005). The mechanisms and molecules involved in these adaptive learning processes, which are often termed inhibitory learning (Bouton and Bolles, 1979; Bouton, 1993; Barad, 2005; Myers and Davis, 2007), are not fully understood, although both ionotropic and metabotropic glutamate receptors have been implicated (Walker et al., 2002; Callaerts-Vegh et al., 2006; Kim et al., 2007; Fendt et al., 2008).

In this study, we tested whether mGluR5 plays a role in inhibitory learning processes using newly generated mGluR5 knock-out mice (supplemental Fig. 1, available at [www.jneurosci.org](http://www.jneurosci.org) as supplemental material). Toward this end, we tested mGluR5 knock-out mice in the acquisition and reversal of an associative conditioned task and a spatial reference task. In the classic pavlovian fear conditioning test, we found that mGluR5 null mice were impaired in the acquisition of fear conditioning, confirming a role for this receptor in neural circuits required for this form of learning (Lu et al., 1997; Rodrigues et al., 2002). Even more strikingly we observed a complete deficit in the ability of mGluR5 knock-out mice to extinguish the fear association in both a tone-cued or context-cued test. In the MWM test, mGluR5 knock-out mice exhibited mild deficits in the rate of acquisition of the regular water maze task. However, when the task was reversed and mice were compelled to learn a new location for the escape platform, mGluR5 knock-out mice performed poorly in this novel situation. Together these findings demonstrate a significant role for mGluR5 in adaptive processes that underlie inhibitory learning.

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## Materials and Methods

All experiments were approved by the Institutional Animal Care and Use Committees of the Salk Institute for Biological Studies.

**Generation of mGluR5 knock-out mice.** Standard gene targeting techniques were used to generate mutant floxed mGluR5 mice (supplemental Fig. 1, available at [www.jneurosci.org](http://www.jneurosci.org) as supplemental material). Genomic DNA for mGluR5 was cloned from a phage library of 129 SVJ mouse genomic DNA fragments (Stratagene). A cassette containing a neomycin resistance (*neo*) gene, flanked by loxP sites, under the control of the phosphoglycerol kinase (PGK) promoter was introduced into the intron 720 bp downstream of exon 7 (supplemental Fig. 1a, available at [www.jneurosci.org](http://www.jneurosci.org) as supplemental material). R1 embryonic stem cells (Nagy et al., 1993) were electroporated with the linearized targeting construct, maintained under G418 positive selection, and screened by Southern blot analysis for homologous recombination (supplemental Fig. 1b, available at [www.jneurosci.org](http://www.jneurosci.org) as supplemental material). Chimeric animals produced by injection of these cells into C57BL/6 blastocysts were bred with C57BL/6 mice, and germ-line transmission of the mutation was assessed by PCR and Southern blot. The *neo* cassette was removed in mice by crossing with transgenic mice with Cre recombinase under control of the protamine promoter (O’Gorman et al., 1997) to produce the floxed mice mGluR5<sup>loxP/loxP</sup> (supplemental Fig. 1c–e, available at [www.jneurosci.org](http://www.jneurosci.org) as supplemental material). Finally mGluR5<sup>loxP/loxP</sup> mice homozygous for the conditional allele were crossed to protamine-cre mice to generate the mGluR5 knock-out mice (mGluR5<sup>del/del</sup>).

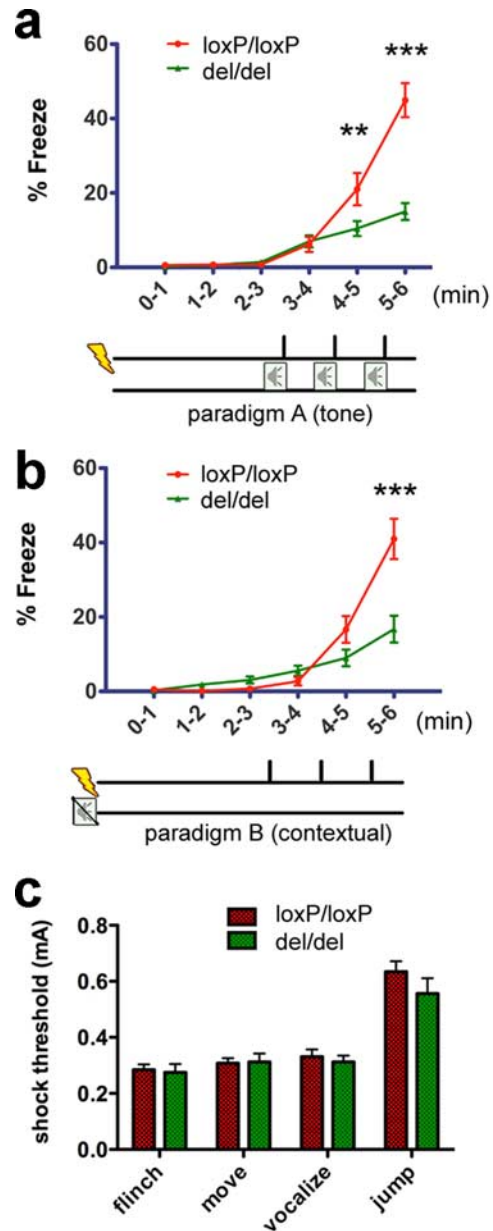
Same sex littermates were housed 2–5 per cage, and maintained at 22°C, with a 12 h light/dark cycle. mGluR5<sup>loxP/loxP</sup> and mGluR5<sup>del/del</sup> mice used in this study were produced by heterozygous breeding of mGluR5<sup>loxP/del</sup>, or heterozygous breeding of mGluR5<sup>loxP/+</sup> and mGluR5<sup>del/+</sup>. Age (2- to 4-month-old) and gender-matched littermates were used for behavioral studies.

**Fear conditioning.** An automated video tracking system was used to monitor mice in the fear conditioning paradigm (Med Associates). Samples were collected at the rate of 30 frames/s. A freezing event was registered only when activity was below motion threshold (20 arbitrary units) for at least 0.5 s. Mice were handled daily for 1 week before the test and were transferred to a holding place adjacent to the testing room for 20 min to 1 h on the day of the test. The training on day 0 (d0) lasted for 6 min. Each mouse was subjected to either training paradigm A, that consisted of 3 min of baseline monitoring, followed by 3 pairs of 20 s tone (85 db, 2900 Hz) coterminated with 1 s footshock (0.7 mA) given at 1 min intervals (Fig. 1a), or paradigm B, which was similar to the paradigm A except that the 1 s footshock was presented without the tone (Fig. 1b).

For contextual memory testing each mouse was returned to the same chamber with the exact contextual settings, but without tone and footshock and monitored for 6 min. Chambers were cleaned with isopropanol between each set of mice (Fig. 2a, d1, d15). For the extinction of contextual memory the same procedure was repeated daily for 10 consecutive days (Fig. 3).

For testing auditory fear (Figs. 2a, d2, d16; 4), the conditioning chamber was altered by covering the grid floor and the three sides with patterned plastic boards, and the top with colored paper. New visual cues were provided on the inside wall of the insulating box and on the walls of the testing room. The conditioning chamber was also scented with Windex and vanilla solution. Windex was used for cleaning after each testing. The testing lasted 6 min for each session. For the experiments presented in Figure 2 (d2 and d16) each mouse was placed into the chamber for 3 min and three sets of 20 s tones were presented at 1 min intervals. For the extinction of auditory fear conditioning each mouse was put into the chamber for 3 min before the 3 min tone was presented. The same procedure was repeated daily for 16 consecutive days (Fig. 4).

**Morris water maze.** The Morris Water Maze experiments were conducted as previously described with some modifications (van Praag et al., 2005; Zhang et al., 2008). A white plastic water tank of 120 cm diameter was filled with water at room temperature. The water was made opaque with white nontoxic Crayola washable paint. A transparent platform (8 × 13 cm) was submerged 1 cm below the surface of opaque water. An automated video tracking system (Ethovision; Noldus Information



**Figure 1.** Deficits in fear acquisition in mice lacking mGluR5. **a**, Fear acquisition by three footshocks paired with tones. mGluR5<sup>del/del</sup> mice ( $n = 28$ ) froze significantly less than mGluR5<sup>loxP/loxP</sup> mice ( $n = 27$ ) ( $F_{(1,106)} = 13.14$ ,  $p = 0.0006$  for genotype;  $F_{(2,106)} = 33.93$ ,  $p < 0.0001$  for training–genotype interaction; Bonferroni post-tests for genotype,  $p < 0.05$  at 4–5 min,  $p < 0.0001$  at 5–6 min). **b**, Fear acquisition by three footshocks. There was less freezing in mGluR5<sup>del/del</sup> mice ( $n = 20$ ) than mGluR5<sup>loxP/loxP</sup> mice ( $n = 25$ ) ( $F_{(1,86)} = 6.31$ ,  $p = 0.0158$  for genotype;  $F_{(2,86)} = 14.48$ ,  $p < 0.0001$  for training–genotype interaction; Bonferroni post-tests for genotype,  $p < 0.0001$  at 5–6 min). **c**, Acute pain threshold to footshock was not altered in mGluR5 null mice. We measured the minimal currents required to elicit four stereotyped reactions against footshock: flinch, move, vocalization, and jump. mGluR5<sup>del/del</sup> mice ( $n = 8$ ) and mGluR5<sup>loxP/loxP</sup> mice ( $n = 13$ ) showed the same pain sensitivity to an increasing electric footshock ( $t$  test,  $p > 0.05$  for each reactions between genotypes). Data are presented as SEM (\*\* $p < 0.01$ , \*\*\* $p < 0.001$  for Bonferroni post-tests).

Technology) was used to record the swim path, velocity and time taken to reach the platform (latency) or the time spent in each zone. The water maze procedure consisted of three phases: (1) visible platform training; (2) hidden platform training and probe test 1; and (3) reversed platform and probe test 2.

Mice were first trained to find the visible platform for 3 d (three trials per day). A thin black plastic brick (8 × 13 × 1.5 cm) was placed above

the transparent platform and a flag was installed 10–15 cm above the surface, allowing mice to visualize the location of the platform. Mice were released from the Southwest (SW) quadrant for all trials. The platform was rotated from the Northeast (NE) to Northwest (NW) to Southeast (SE) quadrant for each trial. Upon release, each mouse was allowed a maximum of 60 s to find the visible platform. Mice that failed to find the platform within 60 s were placed onto the platform. The mouse was allowed to remain on the platform for 15 s after each trial.

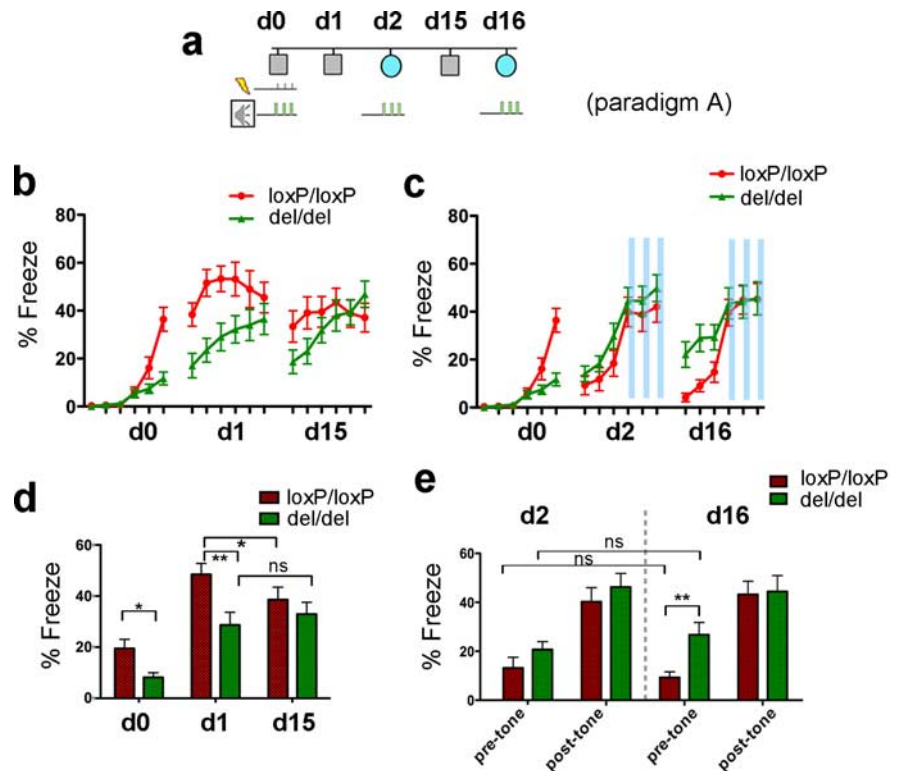
Hidden platform training was conducted one day after the completion of the visible platform training. Mice were trained for 7 d (three trials per day) to find the submerged platform at a fixed position (center of NE quadrant) without any visible local cues. Distal cues in the testing room, such as a computer desk and patterned cardboard on a white wall, were provided as spatial references. Each trial lasted either until the mouse found the platform, or for 60 s. Starting points were changed every trial. Mice were allowed to rest on the platform for 15 s after each trial. The first probe trial was administered 24 h after the last trial of the hidden platform training. During the probe trial the mouse was allowed to swim for 60 s without the platform in the tank.

For the reverse platform training, the hidden platform was moved from the NE quadrant to the center of the SW quadrant without changing any distal visual cues. Mice were then trained to find this new platform location for 4 d (three trials per day). Day 1 of reverse training was conducted 2–3 h after the first probe test. Starting points were changed every trial. The second probe test was performed 7 d after the final training.

**Statistical analysis.** Statistical analyses were conducted with Graphpad Prism. For the multiple trial experiments including fear extinction tests and MWM tests, Two-way repeated-measures ANOVA was conducted to assess the effects of both genotype and sessions/trials. Bonferroni *post hoc* tests were conducted to compare genotype effects at individual session/trial (Figs. 3*b*, 5*a,b*, 6*a–c*). Repeated measures one-way ANOVA were also conducted to assess the effects of training blocks/trials within the same genotype group. Dunnett's multiple comparison post-test was also performed to compare the means at individual time points to the control (first trial/block) (Fig. 6*a,b*). Differences between two means presented in Figure 2*d–e* were assessed with *t* tests. Post-test for linear trend was performed to determine whether there is an increasing/decreasing trend in fear response during fear extinction tests (Figs. 3, 4). Data are presented as mean  $\pm$  SEM. Differences were considered significant if *p* values were  $< 0.05$ .

## Results

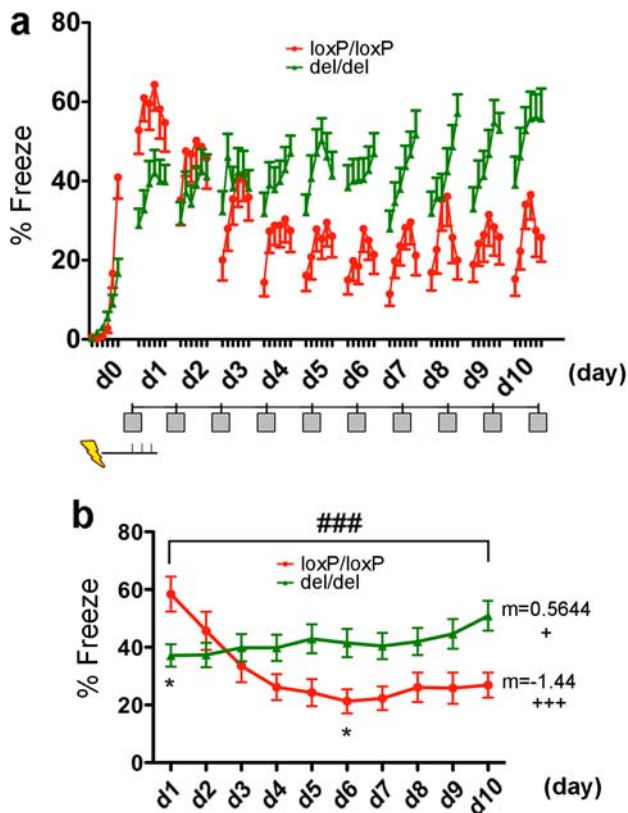
To test whether mGluR5-deficient mice demonstrate memory or learning impairments we performed two learning and memory tasks: pavlovian fear conditioning and the Morris Water Maze (MWM) test. Pavlovian fear conditioning is a task in which a fear response to a neutral conditioned stimulus (CS) is learned when the CS is repeatedly paired with an aversive unconditioned stimulus (US) (LeDoux, 2000). In these experiments, the CS was either a context-specific environment or a loud tone (85 db, 2900 Hz) that was coupled to the US (footshock, 0.7 mA). To deter-



**Figure 2.** Effects of deleting mGluR5 on both contextual and tone-cued fear conditioning. *a*, The experimental paradigm. Mice were trained at d0 by three footshocks paired with tones in context 1 (marked by gray box), returned to the same context at d1 and d15 for 6 min without tone presentation. At d2 and d16, mice were put into different context (marked by light blue circle) for 6 min, with three pairs of 20 s tone presentations of 1 min intervals starting at 3 min. *b*, After fear acquisition at d0, context-cued tests were conducted at d1 and d15, respectively. Freezing was scored at 1 min intervals (mGluR5<sup>del/del</sup>, *n* = 17; mGluR5<sup>loxP/loxP</sup>, *n* = 18). *c*, Auditory fear conditioning tests were conducted at d2 and d16, respectively. Freezing was scored at 1 min intervals (mGluR5<sup>del/del</sup>, *n* = 17; mGluR5<sup>loxP/loxP</sup>, *n* = 18). Time periods for tone presentations were highlighted with blue bars. *d*, Freezing in (*b*) was scored at 6 min intervals. mGluR5<sup>del/del</sup> mice exhibited  $< 50\%$  postshock freezing (d0) compared with mGluR5<sup>loxP/loxP</sup> mice ( $p = 0.0104$ , *t* test). In the first context test (d1) performed 24 h after training, mGluR5<sup>del/del</sup> mice showed a 40% reduction in the context-cued freezing time ( $p = 0.0047$ , *t* test). The ratios of postshock freezing in d0 versus contextual freezing in d1 were comparable between the two groups ( $0.465 \pm 0.1154$  and  $0.3830 \pm 0.1123$  for mGluR5<sup>loxP/loxP</sup> and mGluR5<sup>del/del</sup> respectively;  $p > 0.05$ , *t* test). Contextual fear in the second test (d15) was slightly reduced in mGluR5<sup>loxP/loxP</sup> mice (d1 vs d15; paired *t* test,  $p = 0.047$ ), but was slightly increased in mGluR5<sup>del/del</sup> mice (d1 vs d15; paired *t* test,  $p > 0.05$ ). *e*, Freezing in (*c*) was scored at 3 min intervals (for pre-tone fear and post-tone fear, respectively). Pretone freezing in the second auditory fear conditioning test (d16) was 2.5 times greater in mGluR5<sup>del/del</sup> mice than the control group ( $p = 0.0028$  *t* test). A significant difference was not detected between other genotype comparisons. Pretone freezing was reduced in mGluR5<sup>loxP/loxP</sup> mice but was elevated in mGluR5<sup>del/del</sup> mice (d2 vs d16), but the differences were not significant. Data are presented as SEM (\* $p < 0.05$ , \*\* $p < 0.01$ , for *t* tests).

mine whether ablation of mGluR5 affects acute acquisition of fear, animals were presented with three pairings of the CS and US (Fig. 1*a*, paradigm A). mGluR5<sup>loxP/loxP</sup> mice displayed immediate postshock freezing that progressively increased after repeated presentations of CS-US. mGluR5<sup>del/del</sup> mice froze after footshocks, but the increment in freezing behavior was significantly lower in comparison with the control group upon repeated trials (Fig. 1*a*). mGluR5<sup>del/del</sup> mice were also impaired in a second training paradigm in which mice were given three footshocks without the presentation of the tone (Fig. 1*b*, paradigm B). Thus, acquisition of fear is impaired in mice lacking mGluR5. This impairment is unlikely to be caused by reduced pain sensitivity to electric footshock because mGluR5<sup>del/del</sup> mice showed the same pain response as mGluR5<sup>loxP/loxP</sup> mice (Fig. 1*c*).

Next, we tested whether mGluR5<sup>del/del</sup> mice retained contextual cued and auditory fear memories. In the first context test performed 24 h after training (d1) in paradigm A, mGluR5<sup>del/del</sup> mice showed a significant reduction in the context-cued freezing



**Figure 3.** Contextual fear extinction was abolished in mGluR5-deficient mice. **a**, Extinction of contextual fear. Mice [mGluR5<sup>del/del</sup>, *n* = 20 (d1–d9), *n* = 14 (d1–d10); mGluR5<sup>loxP/loxP</sup>, *n* = 25 (d1–d7), *n* = 15, (d1–d10)] were trained in d0 with three footshocks and returned to the same context (marked by gray box) up to 10 consecutive days for 6 min each day. Freezing was analyzed at 1 min intervals. During extinction training (d1–d10), mGluR5<sup>loxP/loxP</sup> mice generally showed bell-shaped time–response with highest freezing occurring at 3–4 min, whereas mGluR5<sup>del/del</sup> mice showed a trend of increase freezing toward the end of 6 min training session. **b**, Freezing in **a** was scored at 6 min intervals. mGluR5<sup>del/del</sup> mice displayed no extinction over the trials (nonmatching two-way ANOVA,  $F_{(1,394)} = 21.26, p < 0.0001$  for genotype;  $F_{(9,394)} = 4.22, p < 0.0001$  for day–genotype interaction; Bonferroni post-tests for genotype,  $*p < 0.05$  at d1 and d6). For the mGluR5<sup>loxP/loxP</sup> group, one-way ANOVA detected significant effect of training ( $F_{(9,210)} = 6.644, p < 0.0001$ ). Post-test further detected significant trend of fear extinction (slope =  $-1.44, p < 0.0001$ ). For the mGluR5<sup>del/del</sup> group, effect of training was not significant by one-way ANOVA ( $F_{(9,184)} = 0.61, p > 0.05$ ). However, post-test for liner trend detected a significant positive trend (slope =  $0.57, p = 0.036$ ). Data are presented as SEM ( $*p < 0.05$ , for Bonferroni post-tests;  $###p < 0.001$  for two-way ANOVA). (Linear slope is denoted by “m”;  $+p < 0.05$  and  $+++p < 0.001$  for ANOVA post-test for linear trend). ns, Not significant.

time (Fig. 2*b*, in d1). The fractions of context-cued freezing were  $0.49 \pm 0.04$  and  $0.29 \pm 0.05$  for mGluR5<sup>loxP/loxP</sup> mice and mGluR5<sup>del/del</sup> mice respectively (Fig. 2*d*) ( $p = 0.0047, t$  test). However, the ratio of freezing during acquisition (d0) to d1 was comparable between the two groups ( $0.47 \pm 0.12$  and  $0.38 \pm 0.11$  for mGluR5<sup>loxP/loxP</sup> and mGluR5<sup>del/del</sup> respectively;  $p > 0.05, t$  test), suggesting that although mGluR5<sup>del/del</sup> mice are impaired in the initial fear acquisition, they retain the ability to express the once-memorized fear response. In contrast to this context-specific deficit, we found that 24 h later (d2) when mice were subjected to the CS tone-cued test, both groups showed similar pretone freezing (Fig. 2*c,e*; 0–3 min in d2) and post-tone freezing (Fig. 2*c,e*; 4–6 min in d2). Therefore mGluR5<sup>del/del</sup> mice are able to remember the association between the CS and US 2 d after training. To determine the retention of these memories at later time points, mice were tested again after 2 weeks (d15 and d16). Contextual fear (d15) was slightly reduced in mGluR5<sup>loxP/loxP</sup>

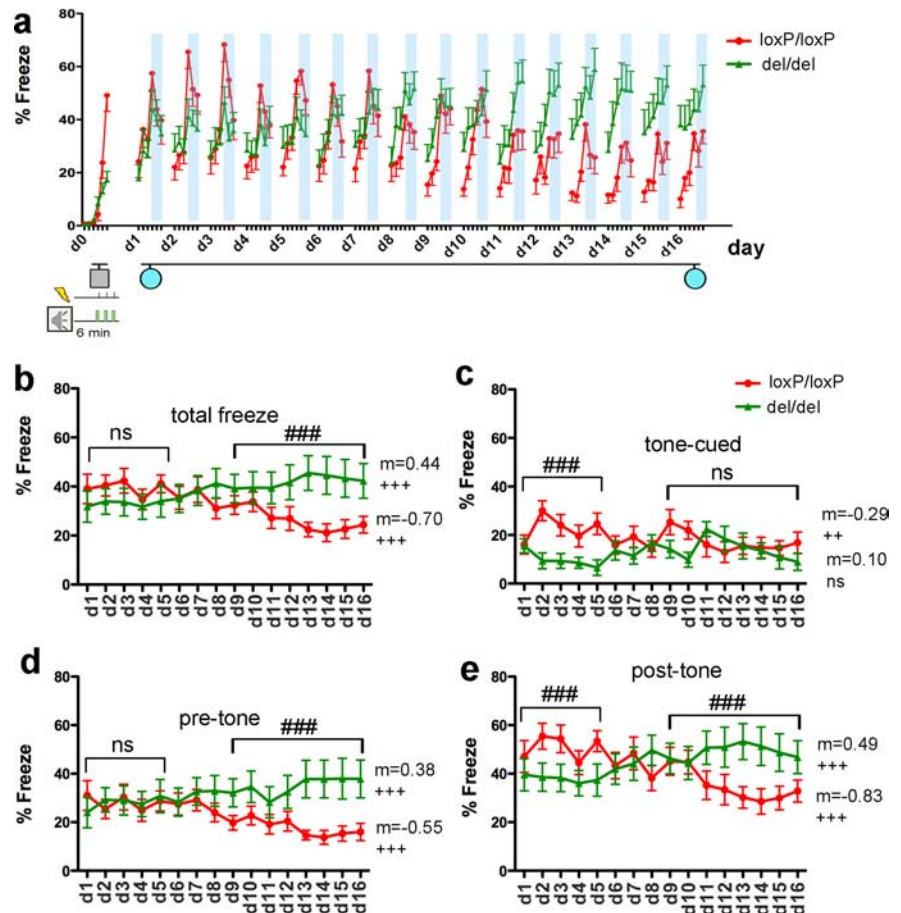
mice (paired *t* test,  $p = 0.044$ ) but was slightly increased in mGluR5<sup>del/del</sup> mice (paired *t* test,  $p > 0.05$ ) (Fig. 2*b,d*, compare d1, d15). In the second CS tone-cued test conducted in d16, both groups displayed similar post-tone freezing (Fig. 2*c,e* in d16). Noticeably, however, pretone freezing (0–3 min) was reduced in mGluR5<sup>loxP/loxP</sup> mice but was elevated in mGluR5<sup>del/del</sup> mice, although these differences were not significant (Fig. 2*e*, compare d2, d16). Pretone freezing in the second auditory fear memory test was 2.5 times greater in mGluR5<sup>del/del</sup> mice than the control group (Fig. 2*e*, d16) ( $p = 0.0028, t$  test), suggesting that mGluR5<sup>del/del</sup> mice became less discriminative to the fear environment compared with the control group after training.

A previously learned aversive association can be suppressed or extinguished by repeated presentation of the CS in the absence of the US. With this training the animal learns that the CS no longer predicts the US such that the fear response is “inhibited.” The most widely accepted theory for the neural basis of extinction is that it is a parallel process distinct from fear acquisition and is often termed inhibitory learning (Bouton and Bolles, 1979; Bouton, 1993; Barad, 2005; Myers and Davis, 2007). This is a distinct mechanism from when extinction is performed very shortly after acquisition of the fear association, when it may be a reversal or unlearning process (Myers et al., 2006). To determine whether inhibitory learning requires mGluR5 we performed fear extinction studies. In the first experiment, mice were trained with paradigm B in which they learn to associate the footshock with a context. Mice were then returned to the same context everyday without footshock for 10 consecutive days (Fig. 3*a*). Freezing in mGluR5<sup>loxP/loxP</sup> mice decreased during the extinction training, as revealed by one-way ANOVA ( $F_{(9,210)} = 5.644, p < 0.0001$ ) (Fig. 3*b*). Dunnett’s multiple comparison test revealed a significant difference between day 1 and all subsequent days. In addition, the post-test for linear trend revealed a significant decreasing trend in freezing during the period of extinction training in mGluR5<sup>loxP/loxP</sup> mice (slope =  $-1.438, p < 0.0001$ ). In contrast to this, fear extinction in mGluR5<sup>del/del</sup> mice was completely abolished (Fig. 3*a*). One-way ANOVA comparison did not detect any effects of extinction training for mGluR5<sup>del/del</sup> mice ( $F_{(9,184)} = 0.7882, p > 0.05$ ) (Fig. 3*b*). In fact, mGluR5<sup>del/del</sup> mice exhibited a slightly increased amount of freezing on d10 ( $51 \pm 5.2\%$ ) than on the first day (d1) ( $37 \pm 3.9\%$ ), although the difference did not reach significance (by Dunnett’s post-tests analysis). The post-test for linear trend revealed a significant increasing trend in freezing for mGluR5<sup>del/del</sup> mice (slope =  $0.5644, p = 0.0036$ ). These experiments are the first to demonstrate a substantial role for mGluR5 in contextual fear extinction.

Next, to determine whether a similar deficit might be observed in extinction of auditory fear, we performed a separate series of experiments. Mice were first trained in paradigm A (tone-cued test), and were then tested in a different context without footshock but with tone presentation for 16 consecutive days. There was a clear difference between mGluR5<sup>loxP/loxP</sup> and mGluR5<sup>del/del</sup> animals in the extinction of both pretone and post-tone fear (Fig. 4*a*). To better assess fear behaviors, freezing was analyzed in four categories: total freezing (0–6 min) (Fig. 4*b*), pretone freezing (0–3 min) (Fig. 4*d*), post-tone freezing (4–6 min) (Fig. 4*e*) and tone-cued freezing (post-tone freezing minus pretone freezing) (Fig. 4*c*). Although initially mGluR5<sup>del/del</sup> mice showed less post-tone freezing than control mice (Fig. 4*e*), this freezing behavior did not diminish during the course of repeated daily exposure to the tone. The control group showed a clear reduction in post-tone freezing which was significant between days 9 and 16 of extinction training (Fig. 4*e*). Both groups dis-

played similar amount of pretone freezing between days 1 and 9 of training (Fig. 4*d*). However as training progressed there was a steady reduction of pretone freezing in mGluR5<sup>loxP/loxP</sup> mice whereas mGluR5<sup>del/del</sup> mice continued to display the same degree, or increased, pretone freezing during the late stages of extinction training (Fig. 4*d*). To further examine the freezing response as a more direct result of the tone cue, we subtracted pretone freezing from post-tone freezing and presented it as tone-cued fear in Figure 4*c*. At the onset (d1–d5), tone-cued freezing was lower in mGluR5<sup>del/del</sup> mice compared with the control group, which displayed the most robust freezing on the second and third day (Fig. 4*c*). However, tone-cued freezing was progressively reduced in mGluR5<sup>loxP/loxP</sup> group, while it was maintained at a similar level throughout the test in mGluR5<sup>del/del</sup> mice. Near completion of the test (d9–d16), tone-cued freezing was no longer different between the two genotype groups (Fig. 4*c*). Overall, there was a significant trend in the decrease of the fear curve in mGluR5<sup>loxP/loxP</sup> mice (slope =  $-0.29$ ,  $p < 0.0031$ ), but not in mGluR5<sup>del/del</sup> mice (slope =  $0.10$ ,  $p = 0.23$ ) (Fig. 4*c*). Finally, comparing total freezing revealed the opposite trends between mGluR5<sup>loxP/loxP</sup> mice and the mGluR5<sup>del/del</sup> mice: no difference in the early part of the test, and significant differences later (Fig. 4*b*). Together these data demonstrate that mice lacking mGluR5 were not able to extinguish tone-cued fear (Fig. 4*c*) and became less able to discriminate the fear environment (Fig. 4*d*) during the course of extinction training. Notably, even the extinction of pretone freezing in the control group was much slower compared with the extinction of context-cued freezing shown in Figure 3; although it might be expected that pretone freezing should stay at relatively low levels when the mice were exposed to a neutral context. A likely explanation for this is that during the extinction training, the mice learned to associate the neutral environment to tone, which they had previously associated to the US (footshocks). In support of this idea, extinction of pretone freezing correlated very well with the extinction of post-tone freezing for both genotype groups, suggesting that the original neutral environment had become another CS for fear.

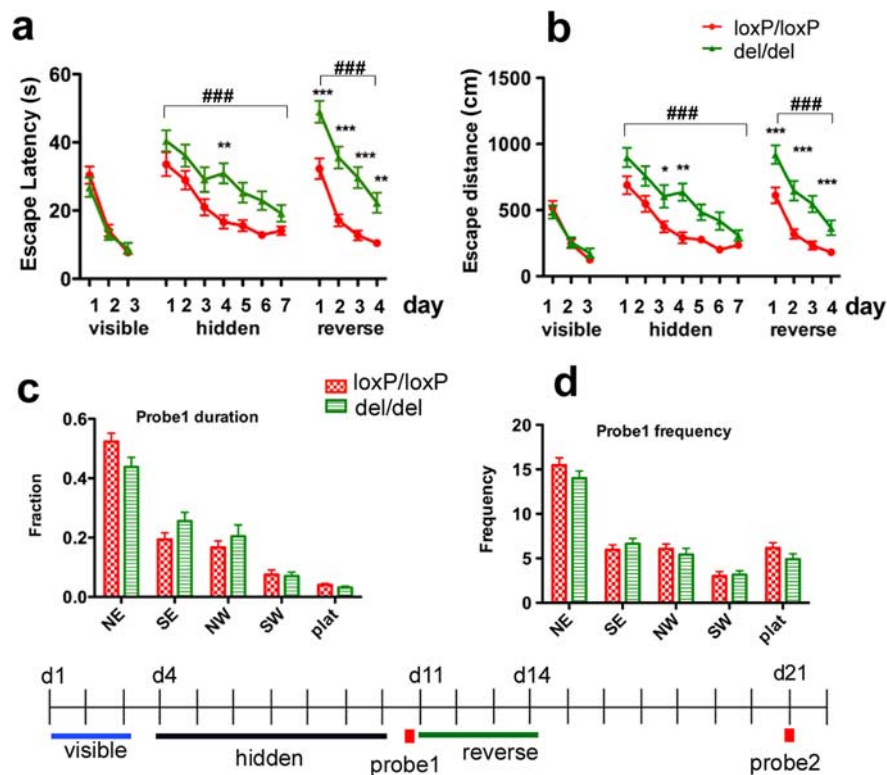
We also conducted a control experiment to examine whether presentation of the 85 db 2900 Hz tone alone, without footshock, could cause freezing in mice. We found that an 85 db tone lasting for 3 min induced little freezing in mice ( $<3\%$  in both groups, see supplemental Fig. 3, available at [www.jneurosci.org](http://www.jneurosci.org) as supplemental material). Therefore the in-



**Figure 4.** Extinction of auditory fear conditioning was abolished in mGluR5-deficient mice. *a*, Extinction of auditory fear conditioning. Mice were trained with three footshocks paired with tones at d0 in one context (gray square) and subsequently returned to a different context (blue circles) for 16 consecutive days. Extinction training on each day lasted 6 min, with one tone of 3 min presented during the final 3 min. Freezing was scored at 1 min intervals to compare within-session fear expression profiles between the two genotype groups (mGluR5<sup>del/del</sup>  $n = 14$ ; mGluR5<sup>loxP/loxP</sup>  $n = 16$ ). Time periods for the tone presentations were highlighted with blue bars (each represents 3 min). Gray square marked for the context for fear acquisition. Blue circles indicate the neutral context where extinction trainings were conducted. *b*, Total freezing in each day analyzed in 6 min block. Initially, mGluR5<sup>del/del</sup> mice ( $n = 14$ ) showed slightly less freezing ( $F_{(1,140)} = 3.86$ ,  $p = 0.0513$ , d1–d5), but this freezing behavior was not diminished during the course of repeated tone presentations. In contrast, mGluR5<sup>loxP/loxP</sup> mice ( $n = 16$ ) showed extinction of auditory fear conditioning upon repeated daily exposure, resulting in less freezing in the second half of testing ( $F_{(1,224)} = 32.64$ ,  $p < 0.001$ , d9–d16). Overall, day–genotype interaction was significant ( $F_{(15,420)} = 7.52$ ,  $p < 0.0001$ ). Post-test detected significant trend of fear extinction in mGluR5<sup>loxP/loxP</sup> mice (slope =  $-0.70$ ,  $p < 0.0001$ ) and significant trend of increase in fear in mGluR5<sup>del/del</sup> mice (slope =  $0.44$ ,  $p < 0.0001$ ). *c*, Extinction of tone-cued freezing. At the onset, tone-cued freezing was less in mGluR5<sup>del/del</sup> mice ( $F_{(1,140)} = 29.57$ ,  $p < 0.0001$ , d1–d5). Near the completion of the test, tone-cued freezing amounts were similar between the two groups ( $F_{(1,224)} = 2.16$ ,  $p = 0.14$ , d9–d16). Two-way ANOVA showed a significant day–genotype interaction from d1 to d16 ( $F_{(15,420)} = 2.80$ ,  $p = 0.0004$ ). Post-test for linear trend detected significant trend of decrease of fear in mGluR5<sup>loxP/loxP</sup> mice (slope =  $-0.29$ ,  $p < 0.0031$ ) but not in mGluR5<sup>del/del</sup> mice (slope =  $0.11$ ,  $p = 0.23$ ). *d*, Pretone freezing analyzed in 3 min block. Although both groups displayed similar amounts of freezing in the early and middle sessions of training (d1–d9), mGluR5<sup>del/del</sup> mice froze more in the late sessions (d9–d16) ( $F_{(1,224)} = 40.15$ ,  $p < 0.0001$ ). Two-way ANOVA showed a significant day–genotype interaction from d1 to d16 ( $F_{(15,420)} = 4.13$ ,  $p < 0.0001$ ). Post-test for linear trend detected significant trend of fear reduction in mGluR5<sup>loxP/loxP</sup> mice (slope =  $-0.55$ ,  $p < 0.0001$ ) and a significant trend of fear increase in mGluR5<sup>del/del</sup> mice (slope =  $0.38$ ,  $p < 0.0001$ ). *e*, Post-tone freezing analyzed in 3 min block. Initially, post-tone freezing was less in mGluR5<sup>del/del</sup> mice ( $F_{(1,140)} = 12.87$ ,  $p = 0.0005$ , d1–d5). However, mGluR5<sup>del/del</sup> mice froze more in the end ( $F_{(1,224)} = 20.2$ ,  $p < 0.0001$ , d9–d16). Two-way ANOVA showed a significant day–genotype interaction from d1 to d16 ( $F_{(15,420)} = 7.50$ ,  $p < 0.0001$ ). A significant trend of fear reduction in mGluR5<sup>del/del</sup> mice ( $m = -0.83$ ,  $p < 0.0001$ ) and a significant trend of fear increase in mGluR5<sup>del/del</sup> mice ( $m = 0.49$ ,  $p < 0.0001$ ) were detected. Data are presented as SEM (###  $p < 0.001$  for two-way ANOVA). (Linear slope is denoted by “ $m$ ”;  $^{+}p < 0.01$  and  $^{+++}p < 0.001$  for ANOVA post-test for linear trend). ns, Not significant.

fluence of this freezing in our experiments is small, and unlikely to confound our conclusions.

In the next series of experiments we determined whether mGluR5<sup>del/del</sup> mice demonstrated a deficit in spatial learning in the standard MWM test. A previous study had found that



**Figure 5.** Deleting mGluR5 impaired performance in the MWM. The water maze procedure consisted of three phases: (1) visible platform training, d1–d3; (2) hidden platform training and probe test 1, d4–d11; and (3) reversed platform and probe test 2, d11–d21. The experimental paradigm is illustrated at the bottom of the figure. **a, b**, Escape times (**a**) and path lengths (**b**) taken to reach the platform. Escape times and path lengths were analyzed by training block (3 trials per day). In the visible platform test, both groups learned the task, as indicated by incremental reductions in escape times ( $F_{(2,72)} = 82.31, p < 0.0001$ ) and distances ( $F_{(2,72)} = 53.91, p < 0.0001$ ) during training. There was no significant difference in either escape times ( $F_{(1,72)} = 0.36, p = 0.5499$ ) or path lengths ( $F_{(1,72)} = 0.07, p = 0.7951$ ) between genotype groups (mGluR5<sup>del/del</sup>,  $n = 19$ ; mGluR5<sup>loxP/loxP</sup>,  $n = 19$ ) in the visible platform test (left block). Mice were then trained to find a hidden platform during the next seven consecutive days (3 trials per day). mGluR5<sup>del/del</sup> mice consistently showed a longer escape latencies and path lengths (middle block) over the training blocks. Repeated-measure two-way ANOVA showed a significant effect of genotype ( $F_{(1,216)} = 15.49, p = 0.0004$ ) and day ( $F_{(6,216)} = 21.59, p < 0.0001$ ) but not of the genotype–day interaction ( $F_{(6,216)} = 0.83, p = 0.5494$ ) on escape latency. Bonferroni post-tests detected significant differences at hidden day 4,  $**p < 0.01$ . Similarly, there were significant effects of genotype ( $F_{(1,216)} = 23.99, p < 0.0001$ ) and day ( $F_{(6,216)} = 26.44, p < 0.0001$ ) but not genotype–day interaction ( $F_{(6,216)} = 1.17, p = 0.3236$ ) on escape length (Bonferroni post-tests for genotype,  $*p < 0.05$  hidden day 3,  $**p < 0.01$ , hidden 4). A probe test was conducted 24 h after the completion of regular platform training (**c, d**). Immediately after the first probe test, the platform was removed to the opposite quadrant in the pool and mice were trained at three trials per day for another 4 d in this reversed setting. Although both groups acquired the task after 4 d training, mGluR5<sup>del/del</sup> mice demonstrated longer escape time (Fig. 5a, right block) ( $F_{(3,108)} = 42.38, p < 0.0001$  for day;  $F_{(1,108)} = 45.68, p < 0.0001$  for genotype;  $F_{(3,108)} = 0.80, p = 0.4978$  for day–genotype interaction; Bonferroni post-tests,  $p < 0.001$  for reverse day 1 and day 3,  $p < 0.01$  for reverse day 4), and path lengths (Fig. 5b, right block) ( $F_{(3,108)} = 36.59, p < 0.0001$  for day;  $F_{(1,108)} = 35.52, p < 0.0001$  for genotype;  $F_{(3,108)} = 0.90, p = 0.4418$  for day–genotype interaction; Bonferroni post-tests,  $p < 0.001$  for reverse day 1, day 2, and day 3,  $p > 0.05$  for reverse day 4). **c, d**, Performances in the first probe test. The first probe test was conducted 24 h after the completion of regular platform training. Analysis of the time spent in the four quadrants (**c**) revealed a significant effect of quadrant ( $F_{(4,144)} = 86.20, p < 0.0001$ ). mGluR5<sup>loxP/loxP</sup> mice spent slightly more time in the target quadrant (NE) and platform zone than mGluR5<sup>del/del</sup>. However, the genotype–quadrant interaction was not significant ( $F_{(4,144)} = 2.24, p = 0.0678$ ). Further analysis of times entered in each testing zone (**d**) also revealed a significant effect of quadrant ( $F_{(4,144)} = 125.02, p < 0.0001$ ). Entries to NE quadrant and platform zone were slightly higher in mGluR5<sup>loxP/loxP</sup> mice compared with mGluR5<sup>del/del</sup> mice, but the genotype–quadrant interaction was not significant ( $F_{(4,144)} = 1.33, p = 0.2615$ ). Data are presented as SEM ( $*p < 0.05, **p < 0.01, ***p < 0.001$  for Bonferroni posttests;  $###p < 0.001$  for two-way ANOVA).

mGluR5 null mice had a robust deficit in performance in this test (Lu et al., 1997). In contrast we found only a mild impairment in performance of mGluR5<sup>del/del</sup> mice in the MWM. During the first 3 experimental days, mice were trained to find a visible platform, and both groups performed equally on this task, suggesting that mutant and control mice could visualize the platform and swim equally effectively (Fig. 5a,b, left). During the next seven experimental days, mice were trained to find a hidden platform (three

trials per day) (Fig. 5a,b, middle). Although both groups acquired the task during training, mGluR5<sup>del/del</sup> mice consistently showed longer escape latencies and path lengths over the training blocks. These disparities were not caused by differences in swim speed. In fact, mGluR5<sup>del/del</sup> mice swam slightly faster during hidden platform training (supplemental Fig. 2, available at www.jneurosci.org as supplemental material). We next performed a probe trial in which the platform was removed from the learned location. The first probe test conducted 24 h after the initial training demonstrated that both groups remembered the location of the platform (Fig. 5c,d). mGluR5<sup>loxP/loxP</sup> slightly outperformed mGluR5<sup>del/del</sup> by spending more time in the target quadrant (NE) and the platform zone (Fig. 5c). However, the genotype-by-quadrant interaction was not significant. mGluR5<sup>loxP/loxP</sup> also made slightly more entries to the target quadrant and the platform zone than mGluR5<sup>del/del</sup> (Fig. 5d), but again, the genotype-by-quadrant interaction was not significant.

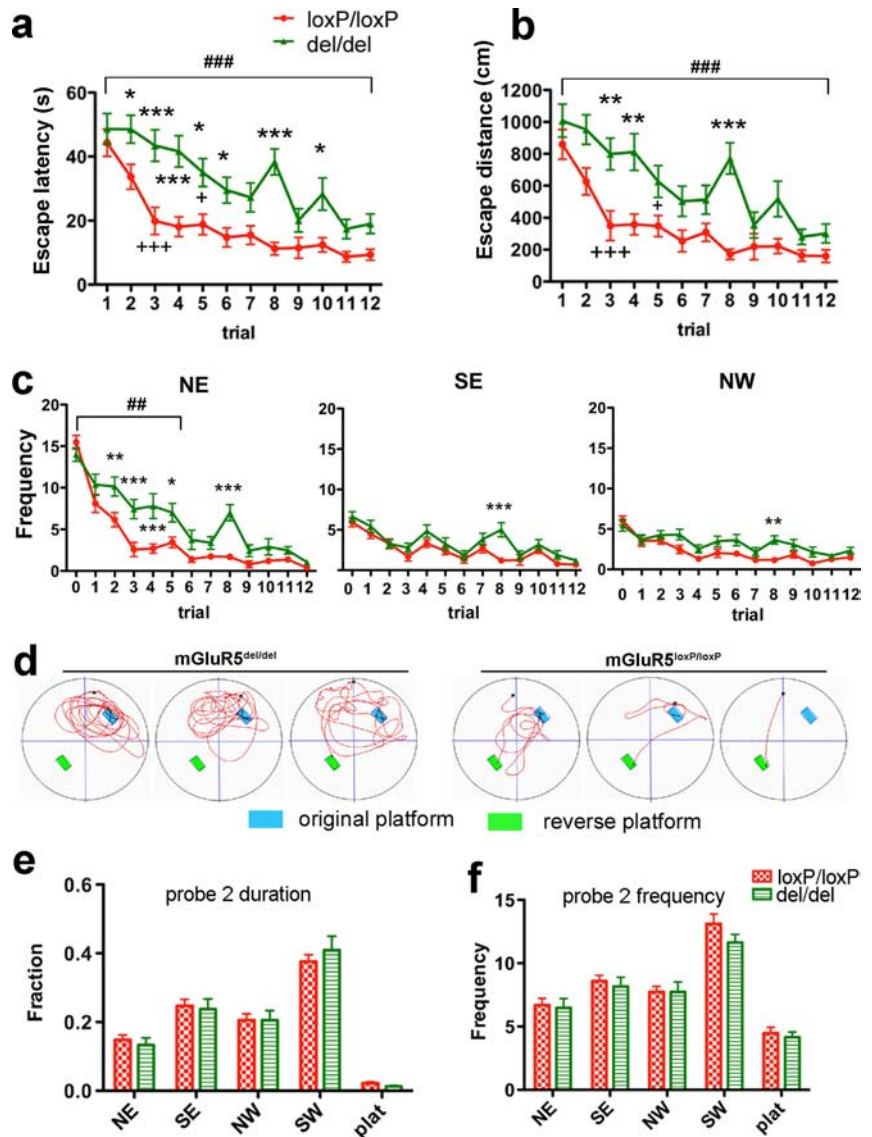
In the final test we performed a reversal of the hidden platform in the MWM. Immediately after probe test 1, the platform was moved to the opposite quadrant location (SW) and mice were trained for 4 d (three trials per day) in this reversed setting. mGluR5<sup>del/del</sup> mice showed significantly longer escape times (Fig. 5a, right) and path lengths (Fig. 5b, right) over the training blocks. When data were analyzed by trials, the group differences were more evident in early training, with significant differences between the genotypes occurring in both latency and distance as early as the second and third trial (Fig. 6a,b). While mGluR5<sup>del/del</sup> mice continued to return to the previous target location, the control cohort more rapidly found the platform in the new location (Fig. 6d, representative tracks taken from trial 2). Throughout this training, mGluR5<sup>del/del</sup> mice entered the NE (previous target location) more often than the control group (Fig. 6c). Meanwhile, the number of entries into the SE and NW (no platform zone) quadrants were much lower for mGluR5<sup>del/del</sup> mice, and were comparable between mGluR5<sup>del/del</sup> and mGluR5<sup>loxP/loxP</sup> mice

(Fig. 6c). These results demonstrate that a persistent search in the previous target zone resulted in a significant delay for mGluR5<sup>del/del</sup> mice to find the new target location. The second probe test in which the platform was again removed was conducted after a further 7 d and revealed no differences in performance between groups (Fig. 6e,f). Thus, spatial memory was intact in mice lacking mGluR5, whereas mice had a clear deficit in the reversal task. These experiments further support a role of mGluR5 in inhibitory learning.

## Discussion

Previous work has highlighted a role for mGluR5 in learning and memory, and in various forms of synaptic plasticity. In the present study we generated a novel strain of mGluR5 mutant mice to test the role of mGluR5 in two common learning paradigms, fear conditioning and the MWM. We found that mGluR5 plays a role in the initial steps of memory acquisition rather than memory storage and retrieval. mGluR5-deficient mice were defective in the acquisition of fear conditioning. However, they retained the ability to express the once-remembered fear response (Fig. 2). Similarly, mGluR5-deficient mice displayed mild impairments in the acquisition of the standard version of the MWM, but were able to remember the location of the hidden platform during the probe test (Fig. 5). Together these findings suggest that mGluR5 mutant mice are defective in the acquisition, rather than the retention and retrieval of the memories. These findings are consistent with prior studies which have demonstrated a role for mGluR5 in the acquisition of fear and spatial memories (Schulz et al., 2001; Rodrigues et al., 2002).

Our study is not the first to generate mGluR5 knock-out mice. The mice used here were generated as conditional allele mutations to eventually allow ablation of mGluR5 in a spatial and temporally restricted pattern. In a prior study a conventional global knock-out allele of mGluR5 was generated (Lu et al., 1997). In behavioral test these mice were impaired in contextual fear conditioning and acquisition of the standard MWM task in agreement with our finding with mGluR5<sup>del/del</sup> mice. In contrast to the prior study, in our experiments we found only mild impairments in the acquisition of spatial learning in the MWM whereas Lu et al., had reported more robust deficits in this form of learning. Notably, in both studies similar deficits were observed in acquisition during the early trial blocks. Knock-out mice displayed progressive learning during these early trial blocks, although their escape latencies were longer than the control groups. The main discrepancy between our findings and the prior study arise in the late training blocks. In the previous study, mGluR5 knock-out mice were unable to improve their performance further in the MWM task in late trial blocks. Their escape latencies leveled at 35–40 s throughout the rest of the training [Lu et al. (1997), their Fig. 5]. In our experiments, our knock-out mice had a steady increase in learning throughout all the



**Figure 6.** Ablation of mGluR5 caused a significant deficit in performance of the reversal task of the MWM. *a, b*, Trial-by-trial analysis of escape times (*a*) and path lengths (*b*) taken to reach the hidden platform at a reversed position. Two-way ANOVA analysis of escape duration showed significant effect for genotype group ( $F_{(1,396)} = 45.68, p < 0.0001$ ), trial ( $F_{(11,396)} = 18.39, p < 0.0001$ ) and trial–genotype interaction ( $F_{(11,396)} = 1.99, p = 0.0284$ ). Dunnett’s multiple comparison test comparing to trial #1 showed that the improvement in mGluR5<sup>loxP/loxP</sup> mice reached significant difference at trial #3 ( $+++p < 0.001$ ). The improvement in mGluR5<sup>del/del</sup> group became significant on fifth trial ( $+p < 0.05$ ). Two-way ANOVA analysis of path lengths also showed significant effect for genotype group ( $F_{(1,396)} = 35.52, p < 0.0001$ ), trial ( $F_{(11,396)} = 15.85, p < 0.0001$ ) and trial–genotype interaction ( $F_{(11,396)} = 1.95, p = 0.0322$ ). Dunnett’s multiple comparison test showed that the improvement reached significant difference on trial #3 ( $+++p < 0.001$ ) for mGluR5<sup>del/del</sup> mice and fifth trial ( $+p < 0.05$ ) for mGluR5<sup>loxP/loxP</sup> mice, respectively. Bonferroni posttests used to compare individual points between genotype groups detected significant differences in escape latency and distance as early as trial 2 and trial 3 (mGluR5<sup>del/del</sup>,  $n = 19$ ; mGluR5<sup>loxP/loxP</sup>,  $n = 19$ ). *c*, Throughout training, mGluR5<sup>del/del</sup> mice entered the NE quadrant (previous target location) more often than the control group ( $F_{(1,180)} = 11.70, p = 0.0016$ , for genotype, trial 0–5;  $F_{(5,180)} = 3.88, p = 0.0023$ , for genotype–trial interaction, trial 0–5) (left). Meanwhile, entries to SE quadrant (trial 0–5, genotype effect:  $F_{(1,180)} = 2.90, p = 0.097$ , genotype–trial interaction:  $F_{(5,180)} = 0.45, p = 0.8164$ ) and NW quadrant ( $F_{(1,180)} = 4.29, p = 0.0455$  for genotype;  $F_{(5,180)} = 1.44, p = 0.2116$  for genotype–trial interaction) were much lower for the mGluR5<sup>del/del</sup> mice, and were more comparable to the control group. Trial 0 represents frequencies entered in each testing zone during probe test 1 from the same group of animals (mGluR5<sup>del/del</sup>,  $n = 19$ ; mGluR5<sup>loxP/loxP</sup>,  $n = 19$ ). Due to an unknown reason, mGluR5<sup>del/del</sup> mice performed poorly in trial 8. They had much longer escape latency (Fig. 6*a*) and distance (Fig. 6*b*) than the control group. Their longer search in trial 8 also resulted in much higher number of entries into all quadrants compared with the control group. *d*, Representative trajectories taken from trial 2, demonstrating that mGluR5<sup>del/del</sup> mice continued to return to the previous target location while control mice more rapidly found the new location. *e, f*, Second probe test performed 7 d after reversed platform training. Both groups remembered the last target location (SW) ( $F_{(4,144)} = 59.61, p < 0.0001$  for time spent in the different quadrants;  $F_{(4,144)} = 52.54, p < 0.0001$  for number of entries). There was no significant genotype–quadrant interaction for time spent ( $F_{(4,144)} = 0.29, p = 0.8815$ ) (*e*) and entry numbers ( $F_{(4,144)} = 0.50, p = 0.7369$ ) (*f*). Data are presented as SEM (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  for Bonferroni posttests; ## $p < 0.01$ , ### $p < 0.001$  for two-way ANOVA; + $p < 0.05$ , +++ $p < 0.001$  for Dunnett’s multiple comparison test).

training blocks. At the end of training, knock-out mice showed similar escape latencies as control group (Fig. 5). While it is unclear how these discrepancies might arise they may be due to differences in experimental protocols or strain differences in the mice used in each study.

The primary novel finding in our study is that mGluR5-deficient mice are impaired in the extinction or reversal of learning. We used two inhibitory learning paradigms to assess a role for mGluR5 in these adaptive learning processes. In the first we found that mGluR5-deficient mice were completely unable to extinguish a previously acquired fear response (Figs. 3–4). In the second we found that mGluR5-deficient mice were impaired in their ability to relearn a reversed platform location after they had previously learned the task (Figs. 5–6).

The genetic components that contribute to fear extinction are not fully understood although a number of molecules have been implicated (Falls et al., 1992; Lin et al., 2001, 2003; Lu et al., 2001; Cain et al., 2002, 2004; Marsicano et al., 2002; Wang et al., 2004; Chen et al., 2005; Ponnusamy et al., 2005; Callaerts-Vegh et al., 2006; Chhatwal et al., 2006; Davis et al., 2006; Kamprath et al., 2006; Burgos-Robles et al., 2007; Kim et al., 2007; Sananbenesi et al., 2007; Sotres-Bayon et al., 2007; Fendt et al., 2008; Hefner et al., 2008) (for a review, see Myers and Davis, 2007). Among these are two members of metabotropic glutamate receptor family: mGluR1 (Kim et al., 2007) and mGluR7 (Callaerts-Vegh et al., 2006; Fendt et al., 2008). Thus, with the results of our study, it appears that mGluRs play a central role in fear extinction.

We also found a previously unknown role for mGluR5 in the reversal task of the water maze. Interestingly, we found that mGluR5 knock-out mice were more impaired in the reversal spatial learning task, than in the initial acquisition of spatial learning task of the regular water maze. Thus, mGluR5 knock-out mice provide yet another example that acquisition of standard spatial learning, and reversal spatial learning can be dissociated by genetic or pharmacological manipulation (Hawasli et al., 2007; Duffy et al., 2008).

The fundamental concept of inhibitory learning, which is determined by the animal learning to suppress a prior learned response is broadly used to describe fear extinction studies (Bouton, 1993; Barad, 2005; Myers and Davis, 2007). The same concept of inhibitory learning has also been applied to reversal of spatial learning (Lattal and Abel, 2001; Varvel et al., 2005; Rossato et al., 2006; Duffy et al., 2008; Labrie et al., 2009). In the reversal task, mice learn that navigating to an area where they had previously learned a platform location is no longer an effective escape strategy. Consequently, mice able to perform this task return to this region with decreasing frequency during the training. Thus although fear extinction and reversal spatial learning are quite separate and distinct tasks they are linked as inhibitory learning tasks which require an active suppression of previously learned associations (Lattal and Abel, 2001; Barad, 2005; Myers and Davis, 2007). Our finding that mice lacking mGluR5 are impaired in both the fear extinction and reversal spatial learning, suggest that these two inhibitory learning paradigms may share common mechanistic properties which involve signaling through mGluR5. There are not many other examples of signaling molecules that have been demonstrated to have similar clear roles in multiple forms of inhibitory learning. However, one such molecule is the CB1 receptor (Marsicano et al., 2002; Varvel et al., 2005). CB1 knock-out mice have significant deficits in fear extinction (Marsicano et al., 2002) and in the reversed MWM task (Varvel et al., 2005) similar to the mGluR5-deficient mice. Interestingly, mGluR5 and CB1 receptors are linked together by their roles in synaptic plasticity. mGluR5 activation is required for endocannabinoid-mediated long-term depression of GABAergic synapses (eCB-LTD) in the hip-

pocampus (Chevalere and Castillo, 2003) and depolarization-induced suppression of inhibition (DSI) in the basolateral amygdala (Zhu and Lovinger, 2005). In particular, the mechanisms of DSI in the basolateral amygdala require mGluR5-mediated release of endocannabinoids and activation of presynaptic CB1 receptors. Therefore it is interesting to speculate that a potential cellular basis for inhibitory learning might be the depression of inhibitory synapses in these brain regions. The findings of our study demonstrate a clear role for mGluR5 in fear extinction and reversal of a spatial task, suggesting that both forms of learning could be linked. However a definitive shared molecular link between these two forms of inhibitory learning will require further experimental clarification.

Neural circuits in several different brain regions play a role in fear extinction and reversal tasks. These include the basolateral nucleus of the amygdala (Falls et al., 1992; Walker et al., 2002), the prefrontal cortex (Quirk et al., 2000; Milad and Quirk, 2002; Burgos-Robles et al., 2007) and the hippocampus (Sananbenesi et al., 2007). mGluR5 is expressed in all these structures, and therefore whether or not mGluR5 signaling in all of these regions, or in specific locales are required for inhibitory learning, remains an open question. In future studies, the novel mice described here will enable us to ablate mGluR5 in specific brain regions to directly address this question, and will provide more detailed information of which circuits are involved in inhibitory learning. Reversal or extinction of previously acquired memories allows animals to adapt to a novel environment or situation. These mechanisms are perturbed in several neuropsychiatric disorders in which traumatic memories persist. Therefore mGluR5 provides a potential target for therapeutic intervention in processes of maladaptive learning.

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