Mice Lacking Metabotropic Glutamate Receptor 5 Show Impaired Learning and Reduced CA1 Long-Term Potentiation (LTP) But Normal CA3 LTP

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Class I metabotropic glutamate receptors (mGluRs) have been postulated to play a role in synaptic plasticity. To test the involvement of one member of this class, we have recently generated mutant mice that express no mGluR5 but normal levels of other glutamate receptors. The CNS revealed normal development of gross anatomical features. To examine synaptic functions we measured evoked field EPSPs in the hippocampal slice. Measures of presynaptic function, such as paired pulse facilitation in mutant CA1 neurons, were normal. The response of mutant CA1 neurons to low concentrations of (1S,3R)-1-amino-cyclopentane-1,3-dicarboxylic acid (ACPD) was missing, which suggests that mGluR5 may be the primary high affinity ACPD receptor in these neurons. Long-term potentiation (LTP) in mGluR5 mutants was significantly reduced in the NMDA receptor (NMDAR)-dependent pathways such as the CA1 region and dentate gyrus of the hippocampus, whereas LTP remained intact in the mossy fiber synapses on the CA3 region, an NMDAR-independent pathway. Some of the difference in CA1 LTP could lie at the level of expression, because the reduction of LTP in the mutants was no longer observed 20 min after tetanus in the presence of 2-amino-5-phosphonopentanoate. We propose that mGluR5 plays a key regulatory role in NMDAR-dependent LTP. These mutant mice were also impaired in the acquisition and use of spatial information in both the Morris water maze and contextual information in the fear-conditioning test. This is consistent with the hypothesis that LTP in the CA1 region may underlie spatial learning and memory.

Key words: hippocampus; gene targeting; long-term potentiation; metabotropic glutamate receptor 5; spatial learning; synaptic function

Recent experiments raise the possibility that G-protein-coupled metabotropic glutamate receptors (mGluRs) may be involved in long-term potentiation (LTP) (Bashir et al., 1993), a potential cellular mechanism for learning and memory (Bliss and Collingridge, 1993). Of the eight mGluRs that have been molecularly characterized (Nakanishi, 1994), those linked to phosphotyrosinylotol turnover (Abe et al., 1992) have been implicated most strongly in LTP (Pin and Duvoisin, 1995). For example, the application of the mGluR agonist (1S,3R)-1-amino-cyclopentane-1,3-dicarboxylic acid (ACPD) was reported to potentiate NMDA- and AMPA-mediated currents directly, and its application during tetanus enhanced LTP (O’Connor et al., 1995). When ACPD was combined with subthreshold tetanic stimulation, or when NMDA and ACPD were co-applied, LTP was induced in the hippocampus (Zheng and Gallagher, 1992). Conversely the mGluR, antagonist (R,S)-α-methyl-4-carboxyphenylglycine (MCPG) inhibited tetanus-induced LTP in both the CA1 region and at the perforant pathway in the dentate gyrus (Bashir et al., 1993; Riedel and Reymann, 1993; Richter-Levin et al., 1994). MCPG only blocked the initial LTP but not LTP arising from subsequent stimulations (Bortolotto et al., 1994). This gave rise to the molecular switch model in which both NMDA receptors (NMDARs) and mGluRs must be co-activated to induce LTP. This is controversial, because other studies showed a lack of effects of MCPG on LTP (Chinestra et al., 1993; Manzoni et al., 1994). The subtype of mGluR involved remains unknown, because these agonists and antagonists are not subtype-specific. Expression studies revealed that the distribution patterns of the group I receptors, mGluR1 and 5, are nearly complementary. For example, in the CA1 area of the hippocampus, mGluR5 is the predominant receptor, whereas mGluR1a is absent (Baude et al., 1993; Shigemoto et al., 1993). mGluR5 is found predominantly in the postsynaptic dendrites and spines of CA1 cells on EM analysis (Romano et al., 1995). One study using gene-targeting techniques has demonstrated that mGluR1 is not required for NMDAR-dependent LTP in CA1 (Conquet et al., 1994), although it modulates mossy fiber and cerebellar LTD (Aiba et al., 1994). To address the involvement of mGluR5, the other group I receptor, we generated mutant mice lacking mGluR5 and report here that NMDAR-dependent LTP is partially impaired in two separate pathways, and this correlates with selective impairments in spatial learning and memory in different tasks. Mossy fiber LTP in CA3, in contrast, is presynaptic and does not rely on postsynaptic NMDARs (Nicoll and Malenka, 1995). This form of LTP was also compared in our mGluR5-deficient mice and was found to be normal. Our study implicates a role for mGluR5 in the regulation of postsynaptic NMDAR-dependent pathways and...
supports the idea that CA1 LTP is necessary for spatial learning and memory.

MATERIALS AND METHODS

**Mutant mice.** A null mutation in the mGluR5 gene was created by gene targeting (Z. Jia and J. C. Roder, unpublished observations) in our 129 strain of R1 embryonic stem (ES) cells (Nagy et al., 1993). Targeted ES clones were aggregated with CD1 morulae, and chimeric males were mated with CD1 females. All mice were derived from F2 litters of crosses between heterozygotes. The LTP phenotype in mGluR5 mutants could not be accounted for by differential inheritance of background genes because: (1) the 129 and CD1 parental lines had equal LTP levels (mean ± SEM) at 63 ± 6% above baseline (n = 22) and 64 ± 5% (n = 36), respectively (Jia et al., 1996); (2) an F2 population from a wild-type mGluR5 × CD1 backcross produced mice with LTP at 69 ± 4% above baseline (n = 36), which is outside the distribution of mGluR5 mutant mice at 50 ± 5% above baseline (n = 19); and (3) a backcross of the mGluR5 mutant 129 chimera to the 129 strain also showed diminished LTP in CA1 (data not shown). Therefore, the loss of mGluR5 shows a similar phenotype in these different mouse strains.

**Neuroanatomy.** Mice were deeply anesthetized with sodium pentobarbital (Somnotil, 80 mg/kg, i.p.) and perfused transcardially with 20 ml of PBS, followed immediately by 40 ml of 4% paraformaldehyde in 0.1 m PBS (pH 7.4). Whole brains were then removed and post-fixed in 4% paraformaldehyde at 4°C for an additional 48 hr. Samples were then placed in a solution of 30% sucrose for 24–36 hr at 4°C. After embedding, 30 μm serial cryostat sections were obtained at intervals of 150 μm for mGluR5+/− and mGluR5−/− as well as wild-type (mGluR5+/+) littermates of similar genetic background and age (45–60 d postnatally). Sections were taken through the entire brain in both the horizontal and sagittal planes (n = 3 animals per genotype) at −20° through the desired region using a Reichert-Jung 2800 Frigocut E cryostat.

**Electrophysiology.** Hippocampal slices (400 μm) were cut from 3- to 7-week-old mice and were placed in a holding chamber for at least 1 hr. A single slice was then transferred to the recording chamber and submerged and superfused with artificial CSF (ACSF, 2 ml/min) that had been saturated with 95% O2–5% CO2 (Gerlai et al., 1995). The composition of the ACSF was (in mM): 124 NaCl, 3 KCl, 1.25 NaH2PO4, 2.5 MgCl2, 2 CaCl2, 26 NaHCO3, 10 dextrose. Field potential recordings were performed with micropipettes at 30 ± 2° filled with ACSF. Synaptic field responses were measured by taking either the slope of the rising phase between 5 and 95% or between 5 and 60% of the peak response. The 5–95% field EPSP (fEPSP) measurement contains both NMDAR- and AMPA-receptor (AMPAR)-mediated components (15 and 85%, respectively), whereas the 10–60% fEPSP measurements contain solely the AMPAR-mediated component. Whole-cell recordings were obtained using the “blind method” and were performed in current-clamp or voltage-clamp mode. The internal pipette solution for voltage clamp contained (in mM) 132.5 cesium gluconate, 17.5 CsCl, 0.05 EGTA, 10 HEPES, 2 MgATP, 2 C6ADP, pH 7.4. For current clamp it contained (in mM) 132.5 potassium gluconate (75 K+–MeSO4, 10 HEPES, 0.2 EDTA, 0.2 NaCl, 2 Mg-ATP, and 0.2 CTP, pH 7.4, 290 mOsm).

Synaptic currents and field potentials were evoked by bipolar tungsten electrodes and recorded with an Axopatch-1D patch clamp amplifier and monitored by computer. Responses were evoked at a frequency of 0.1 Hz in CA1 and dentate gyrus and 1 Hz in the mossy fiber pathway. Tetani to evoke LTP in CA1 consisted of four trains of 100 Hz stimulation lasting 500 msec, at an intertrain interval of 10 sec. LTP in the dentate gyrus was measured in the presence of 100 μM picrotoxin. LTP in mossy fiber CA3 was evoked by one train of 100 Hz stimulation for 500 msec. Tetanus was delivered in the presence of 50 μM D–(−)-2-amino-5-phosphonovaleric acid (APV) to prevent contamination with the NMDA-dependent pathway converging on CA3 neurons. In whole-cell voltage clamps the AMPA-mediated component was taken as the difference between EPSCs recorded in the presence and absence of CNOX 5 msec after the stimulus. The NMDA component was measured in the presence of CNOX 50 msec after the stimulus in medium containing 1.5 mM Mg2+ and 100 μM picrotoxin.

Several studies all mice were bred in our animal colony and were housed in groups of four or five animals in cages (33 × 15 × 12 cm) in a room with an ambient temperature of 20 ± 1°C and 12:12 hr light/dark cycle with lights on at 7 A.M. Animals were tested at the age of 3 ± 0.5 months between 10 A.M. and 4 P.M. The control animals were always littermates of mutants, and both control and mutant groups were sex-balanced.

The open field test. The circular apparatus (for details of design, see Janus et al., 1995) was placed 30 cm above the floor in the center of a 3.05 × 3.05 m room. The illumination in the room was provided by centrally placed in-ceiling fluorescent lights. A mirror (80 × 60 cm) placed at an angle on the wall enabled unobtrusive observation of the behavior of the mice. The observer was present in the room, recording subjects’ behavior on a computer.

All subjects were individually tested in one 5 min session. Each subject was introduced to the apparatus in the same place of the arena near the wall. The following behaviors were recorded: (1) locomotor behavior, measured both as the time (seconds) a subject moved at least half the distance of its body length and the number of fields crossed (the hind legs of the subject crossing a line as the criterion); (2) pause, including brief immobility periods; (3) rearing, a subject body is raised supported only by hind legs and a tail; (4) wall leaning, like rearing, but front legs were touching the wall of the apparatus; and (5) grooming, which included fur cleaning and/or combing and face washing. Locomotor behavior, pause, and rearing were recorded separately for the central and peripheral fields of the arena. Also, a latency of central field exploration (seconds) was recorded.

The water maze. Mice were tested in the hidden and visible platform versions of the test, as described previously (Gerlai et al., 1995). The pool was at least 40 cm from the nearest extramaze cue. The pretraining session began with a subject placed on the platform for 15–20 sec. The animal was then placed in the water and allowed to swim for 15–20 sec, after which it was returned to the platform for another 15 sec. Finally, the mouse was given three trials of climbing the platform. In each of these trials the experimenter placed the mouse gently at the base of its tail from the platform into water and allowed it to climb the platform again. After the pretraining session commenced, the subject was returned into a holding cage for 30 sec before the first training trial began.

Each subject was given six training trials per day. Each trial began by placing the mouse in the water, near and facing the wall of the pool. The subjects were oriented for each subject were chosen randomly from any of the three quadrants other than the one with the platform. Each subject was allowed 60 sec to find the platform. If it failed to reach the platform within that time, it was guided by the experimenter to the platform. Subjects were always allowed a 10 sec rest on the platform. For each subject, the latency to reach the platform was recorded. The subjects that did not reach the platform during a trial were assigned a latency of 60 sec. In the visible platform version of the water maze, the submerged platform was indicated with a black flag (5 × 3 cm), and its location was varied randomly between four possible quadrants of the pool for each subject and trial of a session. In the hidden platform test, the platform location was always in the same position (southeast quadrant).

Contextual fear conditioning. The conditioning chamber consisted of one closed side (21 × 20 × 19 cm) of a shuttle box (Shuttle-Scan SC II, San Diego Instruments, Inc., San Diego, Calif.). The floor of the chamber consisted of stainless steel bars that were connected to a computer, which controlled the duration of a test session, timing, and intensity and duration of shock or sound. Background noise was 52 dB. Single subjects were allowed to explore the chamber for 120 sec before the onset of a discrete conditioned stimulus (CS), which consisted of continuous sound (3600 Hz, 95 dB) lasting 30 sec. During the last 2 sec of this CS period a subject was exposed to an unconditioned stimulus (US), a continuous foot shock (0.75 mA). Approximately 24 hr later, subjects were given a 5 min contextual conditioning test in the same chamber in which they were trained. The CS test (6 min) was performed 2 hr later. During this test the chamber was altered by covering the floor and walls with gray plastic, adding an additional source of light and changing its olfactory characteristics. During the first 5 min of the test the CS was not presented (pre-CS stage), after which subjects were exposed to 3 min of the tone. Fear response was assessed by recording the subjects’ freezing response. Freezing was defined as absence of any locomotor activity, except for respiratory movements, sometimes slight head movements, and occasional tail rattling. The animals were usually in a crouching position. In addition to freezing, the subjects’ locomotion, pause, wall leaning, rearing and grooming were recorded. The chamber was cleaned with 70% alcohol after each subject was tested.

Statistical analysis. Data from males and females and from heterozygous and wild-type mice were pooled together, respectively, because no significant differences were found between these groups on any obtained measure. The behavioral data were analyzed using ANOVA.
with repeated measures for the time factor. In cases of departure from sphericity, in repeated measures of ANOVA, degrees of freedom were reduced by the Greenhouse–Geisser correlation (Stevens, 1992). Whenever multiple, interrelated behavioral measures were analyzed, a multivariate ANOVA (MANOVA) was used (Stevens, 1992). Planned comparisons between control and mutant mice were carried out using \( t \) tests. Only significant results are reported in Results. Electrophysiological experiments (group comparisons in LTP) were performed on 5 min blocks of data within the last 30 min of LTP recording using repeated measures ANOVA. All variance values in the text and figure legends represent SEM.

RESULTS

CNS development is normal

In a previous study we showed that the mGluR5 null allele was inherited in a mendelian fashion, and mGluR5^-/-^ mice showed undetectable levels of mGluR5 protein but normal levels of other glutamate receptors (Z. Jia and J. C. Roder, unpublished observations). Immunocytochemistry using antibodies specific for both mGluR5 isoforms (Minikami et al., 1993) showed no mGluR5 in the hippocampus but normal mGluR1 expression.

To examine mGluR5 mutant mice for potential developmental abnormalities, several sets of serial sections were obtained from mGluR5^-/-^ and mGluR5^+/+^ control littermates. Thionin-stained 10 \( \mu \)m serial paraffin sections were taken through the entire extent of the cranium at 300 \( \mu \)m intervals, in both the sagittal and coronal planes \((n = 3)\), for both mGluR5^-/-^ and mGluR5^+/+^ littermates \((45–60 \text{ d postnatally})\). Thirty micrometer serial cryostat sections were also obtained at intervals of 150 \( \mu \)m for mGluR5^+/+^ and mGluR5^-/-^ mice through the entire horizontal and sagittal planes \((n = 3\) for each plane). As shown for regions known to express mGluR5 (hippocampus, cortex, striatum, and olfactory bulb), mutant mice revealed no significant neurodevelopmental abnormalities for these, or other, structures within the CNS (Fig. 1). In addition, 30 \( \mu \)m serial cryostat sections (coronal plane) of mGluR5^-/-^ and mGluR5^+/+^ mice \((300 \text{ \( \mu \)m intervals})\) were stained for cytochrome c oxidase activity, a gross indicator of the level of presynaptic input. These also showed no significant differences between the two groups \((n = 2\) animals per group) (data not shown). Thus, mGluR5^-/-^ mice were found to be without major neuroanatomical abnormalities, possessing all major neuroanatomical loci and fiber pathways in normal proportions.

mGluR5 is the main high-affinity ACPD receptor in CA1

The mGluR agonist ACPD has been shown to produce several effects, including a reversible depression of synaptic transmission in the hippocampus (Pin and Duvoisin, 1995). In the CA1 area, multiple subtypes of mGluRs have been implicated in the depression of \( \text{fEPSP} \), namely groups I and III (Gereau and Conn, 1995). Because ACPD is not active at group III mGluRs, and carbachol, an agonist for the muscarinic acetylcholine receptor, were compared between mutants and wild-type controls. The depression in \( \text{fEPSP} \) after application of various concentrations of \( \text{L}-2\text{-amino-4-phosphonobutyric acid} \) (AP4), or carbachol, was the same in these two groups, suggesting that functional effects mediated by group III mGluRs and muscarinic acetylcholine receptors was not altered in the mutant mice (Fig. 2D,E).

Altered synaptic responses in mGluR5 mutants

The efficacy of excitatory synaptic transmission in the Schaffer collateral–commissural pathway in the CA1 region of the hippocampus in the mGluR5 mutant and control mice was examined. The amplitude, time course, and current–voltage relations of the AMPA component \((\text{CNQX-sensitive})\) of synaptic currents, obtained with whole-cell recordings, revealed normal responses in the mutant mice (Fig. 3A,B). However, the NMDA receptor channel current, expressed as the ratio to the non-NMDA component, was significantly \((p < 0.01)\) reduced in the mutant slices at the holding potentials between \(-20\) and \(+60\) mV (Fig. 3C), although the NMDA receptors retained their usual voltage dependence (Fig. 3B). The extent of paired pulse facilitation, a measure of presynaptic function, showed no significant difference over an interpulse interval range of \(20–500\) msec (Fig. 3D). Finally, the input–output curves, constructed by plotting the slope of \( \text{fEPSP} \) versus presynaptic fiber–volley amplitude, were identical (data not shown).

Decrease in hippocampal LTP in mGluR5 mutants

To examine the possible consequences of the reduced NMDA currents, we examined the ability of three hippocampal pathways to undergo LTP in mice lacking mGluR5 (Fig. 4). In this series of experiments \( \text{fEPSPs} \) were measured at \(5–95\%\) of the peak, and LTP was induced by four trains of tetani. In the CA1 region, the magnitude of LTP was significantly reduced \((35\%)\) in the mutant slices compared with the wild-type controls. Averaged LTP in the last 30 min after tetanus was \(50.2 \pm 5.7\%\) above the \((100\%)\) baseline from 19 mutant slices \((19\) animals\) and \(77.6 \pm 10.2\%\) above the baseline from 19 wild-type slices \((19\) animals) \([F_{(1,32)} = 6.45; p = 0.016]\) (Fig. 4A). When we measured \( \text{fEPSP} \) in
Figure 1. Neuroanatomy in mGluR5 mice: pairwise comparisons of adult mGluR5\(^{+/−}\) (top panels) and mGluR5\(^{−/−}\) (bottom panels) littermates. Thirty micrometer cryostat sections at comparable levels are shown. A, Parasagittal sections showing the hippocampal formation (right, rostral; left, caudal). B, Horizontal sections through the forebrain and thalamus (2.8 mm ventral to the dorsal aspect of brain). C, Horizontal sections through the hindbrain (4.5 mm ventral to the dorsal aspect of brain). D, Parasagittal sections of the forebrain (300 µm from midline). E, Parasagittal sections showing structures of the diencephalon, hippocampus, and splenium of the corpus callosum (300 µm from midline). F, Parasagittal sections of the caudal aspect of the brain, showing regions of the superior and inferior colliculus and brainstem (450 µm from the midline). Scale bars, A, 500 µm; B–F, 1000 µm. Labeled structures represent regions previously shown to express high levels of mGluR5: c1, Hippocampal subfield CA1; c3, hippocampal subfield CA3; ct, neocortex; dg, dentate gyrus; hp, hippocampus; na, nucleus accumbens; ob, olfactory bulb; s, subiculum; sp, medial septal region (low in mGluR5 expression); str, striatum; tu, olfactory tubercle.
CA1 at 10–60% of the peak, LTP in wild-type mice was 168 ± 14% (19 slices from 19 animals) (Table 1), which was not significantly different \( (p = 0.28) \) than LTP in mutants, 148 ± 11% (19 slices from 19 animals). The slope measurement at 5–95% of the peak, in Figure 4, included both AMPA receptor- and NMDA receptor-mediated components, because addition of 2-amino-5-phosphonopentanoate (AP5) removed ~15% of the potential, whereas CNQX removed 85% of the potential. Therefore, about half of the missing LTP in CA1 may be accounted for at the level of induction. The other missing portion may lie at the level of expression, because subsequent experiments in 50 μM AP5 20 min after LTP induction removed some of the difference between wild-type and mGluR5 mutant mice (Table 1). These data indicate that the NMDA receptor-mediated component contributed significantly to the field EPSP, and the reduced LTP recorded with fEPSP in mGluR5 mutants was likely attributable to elimination of this component.

A significant reduction in the degree of LTP was also seen in the medial perforant pathway of the dentate gyrus, where the averaged LTP from nine slices (five animals for each genotype) was 40.4 ± 5.7% above baseline for the mutants, compared with 66.9 ± 9.2% for the wild-type controls \( [F_{(1,12)} = 6.2; p = 0.028] \) when fEPSP was measured at 5–95% of the peak (Fig. 4B). However, when the mossy fiber LTP in the CA3 region was compared between the mutants and the wild-type controls, we could not detect any significant difference in either magnitude or time course (Fig. 4C). The averaged mossy fiber LTP from nine slices (five animals for both groups) was 60.3 ± 14.7% for mutants and 50.6 ± 5.8% for wild-type controls \( [F_{(1,12)} = 0.31, \text{NS}] \). Therefore, LTP was produced in all three pathways of the hippocampus but was selectively attenuated in CA1 and the dentate gyrus.

### Table 1. Analysis of LTP in CA1 in control and mGluR5 mutant mice using differential slope parameters

<table>
<thead>
<tr>
<th>mGluR5 genotype</th>
<th>5–95% of slope in AP5a</th>
<th>5–95% of slope</th>
<th>10–60% of slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/- Genotype</td>
<td>177.6 ± 10.2; n = 19</td>
<td>164.8 ± 16; n = 8</td>
<td>168 ± 14; n = 19</td>
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<td></td>
<td>( p = 0.41^a )</td>
<td>( p = 0.495^a )</td>
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</tr>
<tr>
<td>+/- Genotype</td>
<td>150.5 ± 5.7; n = 19</td>
<td>149.9 ± 12; n = 8</td>
<td>148 ± 11; n = 19</td>
</tr>
<tr>
<td>+/- vs +/-</td>
<td>( p = 0.016 )</td>
<td>( p = 0.431 )</td>
<td>( p = 0.244 )</td>
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a AP5 (50 μM) was added 20 min after tetanic stimulation.

\( p \) values for a comparison with the first column, +/-, 5–95% slope, four-train tetanic stimulation. \( n \) represents the number of slices. Each mouse yielded one or two slices.

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Figure 2. Decreased response to ACPD in mGluR5 mutants. A. Mean ± SEM depression of fEPSP slope induced by ACPD. Data are expressed as mean percentages of the control values in mutant mice (○), six slices, six animals) and wild-type mice (○, six slices, six animals). Traces taken from representative experiments show the effects of ACPD on evoked fEPSP. Each trace is an average of six sweeps recorded immediately before drug application (0) or after 10 min in the agonist at concentrations of 10 (1), 25 (2), 50 (3), 100 (4), and 300 (5) μM. B, Whole-cell current-clamp recording showing the time course of EPSP depression by bath application of ACPD in a single wild-type (●) or mutant (○) CA1 cell, representative of four cells. The constant current (20 pA) hyperpolarizing pulse preceding the EPSP did not give any evidence of the input resistance changes during ACPD applications. C, Representative time courses of EPSP depression in whole-cell current-clamp recordings in the medial perforant pathway of the dentate granule neurons in the presence or absence of ACPD and 1 mM MCPG in wild-type (●) and mutant (○) mice. Insets in B, C, Representative EPSP before (0), during (1), and after (2) ACPD application. D, Mean ± SEM percent fEPSP after L-AP4 application to CA1 from control (●) and mutant (○) mice \( (n = 4) \). E, Mean fEPSP after carbachol addition to control (●) and mutant (○) mice (six slices, three animals).

Descriptive behavioral observations

The mGluR5 mutants were indistinguishable in their weight and morphology. No changes in locomotor and other behaviors were apparent in home cages. When moved to new cages, mGluR5 mutants were soon engaged in normal exploratory behavior, saw-
dust, digging, rearing, and cage top climbing. Mutant males were also engaged in occasional bursts of fighting, at the same rate as males in control groups. However, mGluR5 mutants were unusually reactive to handling. They were difficult to catch in their home cage and often jumped off the experimenter's hand or off the cage. This reactivity to handling showed slow habituation. To ensure a blind experiment all cages were coded, and handling and behavior recording were carried out by different experimenters.

**Behavior in the open-field test**

To examine motility and response to a novel environment, control and mutant mice were observed in an open-field test. Because no differences were found between fields crossed and time of walking or between behaviors in the periphery and center of the arena, we removed field crossing from the analysis and pooled peripheral and central field data together. The analysis of behaviors (walking, pausing, wall leaning, rearing, and grooming) performed by mice did not reveal any significant differences between groups [MANOVA, F(5,16) = 0.25; p = 0.93] (Table 2). In addition, the mGluR5 mutants did not differ from controls in their latency of central field exploration \([t(9) = 0.33; p > 0.05]\). The results indicated that mGluR5 mutant mice did not differ from control

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**Figure 3.** Reduced NMDA component of synaptic transmission in hippocampal slices from mGluR5 mutant mice. A, The EPSC traces were recorded from CA1 neurons in whole-cell voltage-clamp mode and were averages of six successive sweeps before (0) and 20 min after (1) the addition of 10 μM CNQX. The holding membrane potentials are indicated between the traces. B, Averaged amplitudes of AMPA- and NMDA-mediated responses in mGluR5 \(^{+/+}\) (●) and mGluR5 \(^{-/-}\) (○) mice, normalized to the 5 msec peak of AMPA EPSC at −80 mV, which was 342.6 ± 38 pA (n = 10) in control and 318.8 ± 34 pA (n = 11) in mutant mice. The AMPA component at −80 mV was taken as 100%, and all other current amplitudes were scaled and expressed as a percentage of the AMPA current. C, Data in B shown as the NMDA component of EPSCs that differed between wild-type (hatched bars) and mutants (open bars). *Significant difference (p < 0.01, t test). D, Magnitude of the paired pulse facilitation of fEPSPs in the CA1 area of the two groups (six slices from three animals for each genotype). P1, First response; P2, second response applied at the indicated intervals on the x-axis.

**Figure 4.** Reduced LTP in NMDA-dependent pathways in mGluR5 mutant mice. The mean ± SEM of the 5–95% slope of the fEPSP, normalized with respect to 10 min immediately preceding the tetanus (●) for hippocampal slices obtained from control (●) or mutant (○) mice in area CA1 (A), the dentate gyrus medial perforant pathway (B), and the CA3 mossy fiber pathway (C). LTP in CA1 was induced by four trains of 100 Hz tetanic stimulation. LTP in the dentate gyrus was induced by four trains of tetanus in the presence of 100 μM picrotoxin. LTP in CA3 was induced by one tetanic train in the presence of 50 μM AP5. Representative traces (average of six sweeps) of fEPSP obtained immediately before (0) and 60 min after (1) the tetanus are shown for a control mouse (a) and mutant mouse (b), respectively.
animals in their locomotor and exploratory behavior in the new environment.

**Decreased learning acquisition in mGluR5 mutants in the water maze**

Control (n = 27) and mGluR5 mutant (n = 22) mice were trained for 6 days in the hidden platform version of the water maze with six trials per day. Analysis of three-trial blocks revealed a significant difference in learning between control and mutant mice \( F_{(1,47)} = 20.45; p < 0.001 \) and between days \( F_{(3,235)} = 28.43; p < 0.001 \). Also, group by day and group by block interactions were significant \( F_{(3,235)} = 3.88; p < 0.01 \) and \( F_{(4,47)} = 12.79; p < 0.001 \), respectively. Both interactions indicated a significant difference in learning acquisition between groups. Throughout the experiment mutant mice always had longer latencies in finding the platform, but beyond the third day of training their latencies leveled at 35–40 sec, whereas the control mice showed a steady decrease, reaching an average of 10–15 sec by the end of training (Fig. 5A). Planned comparisons revealed that the most significant differences between groups occurred after the third day of training (sixth three-trial block; Fig. 5A). To elucidate further the nature of the above interactions, an analysis of simple effects with trend analysis and an effect size (Stevens, 1992) was performed for each group. For the control group we found a significant decrease in latencies over time \( F_{(5,286)} = 17.94; p < 0.001 \), with 92% of the variance \( (\eta^2 = 0.916) \) accounted for by the change in learning. Two trends were found significant, linear and quadratic \( F_{(1,26)} = 74.8; p < 0.001 \); and \( F_{(1,26)} = 5.2; p < 0.05 \), respectively. The linear trend accounted for 74% and the quadratic accounted for 17% of the variance in learning acquisition. Accordingly, there was a significant change in learning in the mutant group \( F_{(5,231)} = 5.63; p < 0.001 \), with 69% \( (\eta^2 = 0.688) \) of the variance explained by the change in learning. Again, only linear and quadratic trends were found to be significant \( F_{(1,21)} = 9.5; p < 0.01 \); and \( F_{(1,21)} = 15.9; p < 0.001 \), respectively, accounting for 31% and 43% of the variance. In conclusion, although both groups learned to find the hidden platform, the character of learning change was different in controls (mostly linear) and mutants (mostly quadratic). At the end of the training period, all animals were given one probe trial, in which the platform was removed and the mice were allowed to search the pool for 60 sec. The

![Figure 5](image-url). Performance of mGluR5 mutants was impaired in the water maze. A, mGluR5 mutants and control mice were trained with two blocks of three trials per day (for 6 d) in the water maze. The average time to reach the hidden platform in the pool was plotted against three-trial blocks. A significant difference was found between groups. B, Percentage of time spent searching in each quadrant of the pool during the probe trial given 1 d after the last training trial. Quadrants: 2, target quadrant (southeast); 1, adjacent to the right (northeast); 3, adjacent to the left (southwest); 4, opposite (northwest). Control animals searched selectively, and significantly longer, for the platform in the training quadrant (2) than mutants. C, The average time to reach the visible platform in each three-trial block (2 d of testing) is presented. ANOVA with repeated measures did not reveal any significant differences between mutants and controls. *p < 0.05; **p < 0.01; ***p < 0.001.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Controls (n = 12)</th>
<th>mGluR5 mutants (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking</td>
<td>202.2 ± 12.5</td>
<td>186.2 ± 12.8</td>
</tr>
<tr>
<td>Pause</td>
<td>59.4 ± 14.6</td>
<td>74.3 ± 16.0</td>
</tr>
<tr>
<td>Wall leaning</td>
<td>24.7 ± 3.8</td>
<td>25.9 ± 5.9</td>
</tr>
<tr>
<td>Rearing</td>
<td>3.8 ± 2.5</td>
<td>4.4 ± 1.9</td>
</tr>
<tr>
<td>Grooming</td>
<td>6.2 ± 1.0</td>
<td>5.9 ± 1.3</td>
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Data represent the average total time (sec) ± SEM. See text for detailed descriptions of observed behaviors.
A. Duration of freezing during the training phase of fear conditioning. Mutant mGluR5 and control mice showed a comparable amount of freezing immediately after the foot shock. The solid line indicates the duration of the tone (CS); squares indicate the 2 sec footshock (US). The mice were tested for contextual conditioning 24 hr after training. Mutant mice showed significantly less freezing than controls when re-exposed to the training chamber. C, A control tone (CS) conditioning test was carried out in a new context 2 hr after the context test. Both mutants and controls showed no freezing in a new context and comparable amounts of freezing when a tone (CS) was presented in a new context. **p < 0.01, ***p < 0.001.

Figure 6. Contextual fear conditioning is impaired in mGluR5 mutants. A, Duration of freezing during the training phase of fear conditioning. Mutant mGluR5 and control mice showed a comparable amount of freezing immediately after the foot shock. The solid line indicates the duration of the tone (CS); squares indicate the 2 sec footshock (US). The mice were tested for contextual conditioning 24 hr after training. Mutant mice showed significantly less freezing than controls when re-exposed to the training chamber. C, A control tone (CS) conditioning test was carried out in a new context 2 hr after the context test. Both mutants and controls showed no freezing in a new context and comparable amounts of freezing when a tone (CS) was presented in a new context. **p < 0.01, ***p < 0.001.

B

C

The mice were tested for contextual conditioning 24 hr after training. Mutant mice showed significantly less freezing than controls when re-exposed to the training chamber. C, A control tone (CS) conditioning test was carried out in a new context 2 hr after the context test. Both mutants and controls showed no freezing in a new context and comparable amounts of freezing when a tone (CS) was presented in a new context. **p < 0.01, ***p < 0.001.

** Decreased contextual fear conditioning in mGluR5 mutants

This paradigm (Philps and LeDoux, 1992) allowed us to test: (1) the subjects’ unconditioned response to shock (US), (2) their ability to associate simple pairing between a shock and a discrete stimulus-like tone (CS), and (3) the context in which they received the shock. Because the changes in duration and frequency of freezing were similar, we present only the total time of freezing responses for control mice (n = 15) and mGluR5 mutant mice (n = 15) (Fig. 6). The control and mutant subjects showed a similar freezing response to shock during training, which suggests similar perception and response to aversive foot shock. The two groups were also not different in exploratory behaviors during the training session. In the context test performed 24 hr after training, the mutant mice showed a significant reduction in the freezing time, especially at the beginning of the test (Fig. 6B) [MANOVA, F(4,20) = 8.7; p < 0.01]. Both control and mutant mice showed similar freezing to tone in the CS test carried out 2 hr after the context test (Fig. 6C). The groups also did not differ in other exploratory behaviors during each phase (first 3 min with no CS and second 3 min with CS) of the CS test.

DISCUSSION

Our data show that mGluR5-deficient mice develop normally at a gross neuroanatomical level but exhibit changes in the synaptic responses of hippocampal neurons, which may be associated with deficits in spatial learning and memory.

The responses of hippocampal neurons to class III agonists (L-AP4) or carbachol, which activate muscarinic acetylcholine receptors, were normal. However, the responses to ACPD in CA1 were dramatically reduced in mGluR5 mutants. In contrast, the ACPD effects were not altered in mGluR1-deficient mice (Aiba et al., 1994). Therefore, mGluR5 is perhaps the best candidate to fulfill the role of the ACPD receptor, because it is localized both presynaptically and postsynaptically at CA1 synapses (Romano et al., 1995). In contrast to the CA1 area, the response to ACPD at various concentrations in the perforant pathway of the dentate gyrus was indistinguishable between the wild-type and mGluR5 mutant mice. Because the depression effect of ACPD was completely blocked by addition of MCPG in both groups, it is reasonable to speculate that such an effect is mediated by mGluR1, mGluR2, or additional unknown members of group I mGluRs. Analysis of mGluR1- and mGluR1/5-double-deficient mice will be valuable in distinguishing these possibilities. The inhibitory ACPD
action on mGluR5 may not be relevant to LTP, because LTP was decreased in the dentate gyrus, where mGluR5 did not seem to respond to ACPD.

The results also suggest that the mGluR5 receptor plays an important regulatory role in LTP in NMDA-dependent pathways in the hippocampus. Although it is generally agreed that the induction of LTP in both the CA1 region and dentate gyrus is dependent on activation of NMDA receptors, whether mGluRs are also required in this process has been controversial (Bliss and Collingridge, 1993; Nakamichi, 1994). The pharmacological profile of the effects of various agonists and antagonists of mGluRs suggests that group I mGluRs (mGluR1 and mGluR5) are potential candidates for LTP modulation. Given the fact that mGluR1 is mainly expressed in the interneurons in the CA1 region of the hippocampus, but not in pyramidal neurons (Baudet et al., 1993), its role as a postsynaptic modulator of CA1 LTP is not likely. Indeed, when the mGluR1 gene was mutated, LTP could still be elicited with full strength in NMDA-dependent pathways of the hippocampus but was impaired in NMDA-independent LTP in the mossy fiber synapses on CA3 (Conquet et al., 1994; see Aiba et al., 1994). Although these studies indicate that mGluR1 does not need to be co-activated to induce or modulate LTP in either the CA1 area or dentate gyrus, it does not rule out the involvement of other members of the group I family. The only other known member of group I mGluRs, mGluR5, fits the pharmacological profile and is richly expressed in pyramidal neurons in the CA1 area. It is therefore the best candidate molecule to serve as a modulator in the induction of LTP in CA1, and, indeed, our data show partially reduced LTP in the absence of mGluR5. Similar results were obtained at the perforant pathway in the dentate gyrus. The fact that the mossy fiber LTP was normal in the mGluR5 mutants but severely diminished in the mGluR1 mutants suggests that mGluR5 is important in specifically modulating NMDA receptor-dependent LTP, whereas mGluR1 specifically modulates NMDA receptor-independent LTP. The mechanism by which mGluR5 modulates LTP could involve its regulation of NMDAR function at the induction and/or expression level of LTP. Hence in mGluR5 mutant mice we saw a reduction in NMDA receptor-mediated current in the CA1 area of the hippocampus. IEPSps measured at 5–95% of the slope contained an NMDAR-mediated component (15%), which was removed in AP5. Therefore, about half of the missing LTP in CA1 could lie at the level of LTP induction. The remainder could lie at the level of LTP expression, because addition of AP5 after tetanus reduced LTP and removed part of the difference in LTP between wild-type and mGluR5 mutants. In intracellular studies presented elsewhere we show that the NMDA component of LTP, after tetanus, is missing in mGluR5 mutants, whereas the AMPA component is normal (Z. Jia, Y. M. Lu, and J. C. Roder, unpublished observations).

If LTP is involved in learning and memory, then the mGluR5 mutants should show some impairment in learning and memory tasks, because hippocampal NMDAR-dependent LTP was significantly reduced in the mGluR5 mutant mice. Our results show that indeed mGluR5 mutant mice were significantly impaired in two different spatial learning tasks, which are known to depend on an intact hippocampus (Morris, 1990; Philips and LeDoux, 1992). In the water maze (Morris, 1990), subjects must actively escape from an aversive situation, whereas in contextual fear conditioning the subjects show passive fear response (freezing) in the context in which they previously experienced shock (Philips and LeDoux, 1992). Each of the above paradigms include nonspatial control tests in which nonspatial learning was evaluated. The performance of mGluR5 mutants in all nonspatial control tests was comparable to that of control animals. Because we did not find any changes in motor and exploratory activities of mGluR5, the impairment in their spatial learning seems to be selective. The learning acquisition impairment in the water maze was confirmed in the probe trial. Although controls persistently searched for the platform, spent significantly longer time in the target quadrant, and more often crossed the platform side, the mutants did not stay significantly longer in the target quadrant and never persistently searched the area of the platform site. The longer latencies of mutants were not caused by their floating in water, inactivity near the wall, or thigmotaxic swimming along the wall. Also, their motivation, visual acuity, swimming abilities, and learning of the association between a single cue and the platform position were the same as in control animals when the platform was marked by a flag. Mutants also showed impairment in the fear-conditioning test, which requires the association of background contextual information with the US, but showed normal learning of association between a tone (CS) and a shock (US). Animals from both groups explored a shock chamber during training at the similar rate and responded identically to US. Our results are compatible with the injection of the class I mGluR5 antagonist MCPG into rats, which reduced spatial learning, whereas a class I agonist applied after learning facilitated memory recall (Riedel, 1996).

A variety of other studies have tried to test correlations between LTP and learning and memory (Martinez and Derrick, 1996). Pharmacological, physiological, and surgical perturbations have been performed, but the results are inconclusive. However, all genetic disruptions (nine of nine) that impaired spatial learning and memory also impaired LTP in the Schaeffer collateral pathway to CA1. This includes the genes for lyn (Grant et al., 1992), α-Ca2+-calmodulin kinase II (CaMKII) (Silva et al., 1992), cAMP response element binding protein-α (Bourchuladze et al., 1994), PKC-γ (Abeliovitch et al., 1993), NMDAR2A (Sakimura et al., 1995), CaMKII-D286 (Bach et al., 1995; Mayford et al., 1996), calbindin D-28 (Molinaro et al., 1996), and NMDAR1 (Tsien et al., 1996). Gene deletion systems that excise the NMDAR1 gene only in the CA1 region several weeks after birth also yielded mice that showed impaired LTP and spatial learning and memory (Tsien et al., 1996). Conversely, gene disruptions that did not impair spatial learning and memory did not alter the Schaeffer collateral pathway in the spatial LTP on CA1 cells (Huang et al., 1995; Nosten-Bertrand et al., 1996). This emphasizes the importance of the Schaeffer collateral pathway in learning and memory. Two studies tended to dissociate LTP and learning and memory in the perforant path and mossy fiber pathways of the hippocampus. The disruption of the thy-1 gene disrupted granule cell LTP in the perforant pathway of the hippocampus (Nosten-Bertrand et al., 1996), whereas learning and memory were normal. Disruption of PKA selectively decreased CA3 LTP in the mossy fiber pathway, but learning and memory were also normal (Huang et al., 1995). The above evidence and our own data here, in which mGluR5 mutants showed normal mossy fiber LTP but decreased spatial learning, provide a double dissociation between them. Therefore, the best remaining correlation between LTP and learning and memory resides in the CA1 region. In the absence of perforant paths, or mossy fiber LTP in the system, information relevant to spatial learning could reach CA1 directly from the entrohinal cortex (Huang et al., 1995).
antagonist (APV), which blocked induction of LTP in the hippocampus and seemed to block “spatial” learning but not a simple visual association task (Morris, 1990; Davis, 1992). However, more recent studies show that APV only impairs spatial learning in task-naive animals, whereas subjects pretrained in a spatial task resisted this inhibition (Bannerman et al., 1995). Because both NMDA antagonists APV and (2R, 4R, 5S)-2-aminoo-4,5-(1,2-cyclohexyl)-7-phosphonooxazol acid blocked LTP but failed to block spatial learning in pretrained rats (Saucier and Cain, 1995), the relationship between LTP and spatial learning is not direct. Whether NMDAR is necessary for learning spatial strategies or simply refining motor skills rather than spatial maps requires more work.

REFERENCES


