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G. Suzuki, N. Tsukamoto, H. Fushiki, A. Kawagishi, M. Nakamura, H. Kurihara, M. Mitsuya, M. Ohkubo and H. Ohta
J. Pharmacol. Exp. Ther., October 1, 2007; 323 (1): 147-156.
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Epileptogenesis Is Associated With Enhanced Glutamatergic Transmission in the Perforant Path
A. Scimemi, S. Schorge, D. M. Kullmann and M. C. Walker
J Neurophysiol, February 1, 2006; 95 (2): 1213-1220.
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Medial Perforant Path Inhibition Mediated by mGluR7 Is Reduced After Status Epilepticus

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Medial perforant path inhibition mediated by mGluR7 is reduced after
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lished May 19, 2004; 10.1152/jn.00315.2004. Metabotropic glutamate
receptor (mGluR)-mediated inhibition within the dentate gyrus is
altered after epilepsy. Whether these changes occur during the devel-
opmental period of the disease (i.e., the latent period) has not yet been
investigated. Field excitatory postsynaptic potentials (fEPSPs) were
recorded in the lateral (LPP) and medial perforant path (MPP) simul-
taneously in adult mouse hippocampal slices 3–9 days after pilo-
carpine (PILO)-induced status epilepticus. Genetically manipulated
mice (mGluR8 knockout and mGluR4/8 double knockout) and phar-
macologically selective agonists were used to identify specific mGluR
subtypes affected after PILO. Pharmacological activation of mGluR7
by L-AP4 in both wild-type and mGluR4/8 double knockout mice
selectively reduced fEPSPs in the MPP, but not LPP, and this level of
inhibition was significantly reduced 3–9 days after PILO-induced SE.
Activation of mGluR2/3 reversibly depressed the fEPSP slopes in
both the MPP and LPP, but no alterations were noted after PILO.
mGluR8 activation selectively inhibited evoked responses in the LPP,
but not in the MPP, and this level of inhibition did not change after
PILO treatment. These data suggest that reduced presynaptic inhibi-
tion mediated by mGluR7, but not mGluR2/3 or mGluR8, may play a
role during the latent period in generating hyperexcitability in the
dentate and thereby contribute to epileptogenesis.

INTRODUCTION

Metabotropic glutamate receptors (mGluRs) play key roles
in synaptic transmission and synaptic plasticity at many syn-
apses throughout the brain (Anwyl 1999; Sheng and Kim
2002), but several lines of evidence also suggest a pathological
role for mGluRs in a variety of neurodegenerative disorders,
including epilepsy (Moldrich et al. 2003; Wong et al. 1999).
Because mGluRs can powerfully modulate both excitatory
(Gereau and Conn 1995; Gutierrez 2002) and inhibitory (Se-
myanov and Kullmann 2000; Shigemoto et al. 1996) neuro-
transmission in the brain (Conn and Pin 1997), we asked
whether reduced mGluR function might contribute to dysfunc-
tional synaptic transmission early in the process of epilepto-
genesis caused by pilocarpine (PILO)-induced status epilepti-
cus.

The dentate gyrus "filters" incoming neuronal activity from
the entorhinal cortex to the hippocampus (Behr et al. 1998;
Stringer et al. 1989), and in this way, helps to modulate
neuronal transfer to the hippocampus proper. During epilepto-
genesis, the dentate gyrus undergoes numerous anatomical and

neurochemical changes in rodent models, and several lines of
evidence suggest a role for mGluRs in this change. First,
expression levels of group II and III mGluRs are enhanced in
rat dentate after status epilepticus (SE) (Aronica et al. 1997,
2000). Second, there is evidence for increased group II mGluR
function after PILO that appears to contribute to disinhibition
of dentate granule cells (GCs) (Doherty and Dingledine 2001).
Third, agonists for group II or III mGluRs can be both anti-
convulsive (Attwell et al. 1998; Folbergrova et al. 2001; Yip et
al. 2001) and neuroprotective (Allen et al. 1999; Gasparini et
al. 1999). Finally, studies of mGluR knockout (KO) mice
indicate that mice lacking mGluR4 are more resistant to ab-
sence seizures (Snead et al. 2000), whereas mGluR7-null mice
develop seizures after ~12 wk of age (Sansig et al. 2001).

The mGluR family of proteins is commonly separated into
three groups based on sequence homology, pharmacology, and
signal transduction pathways (Conn and Pin 1997). Eight
receptor subtypes have been identified. Group I mGluRs
(mGluR1 and 5) are coupled via Gq to phospholipase C and
phosphoinositide hydrolysis and act primarily postsynaptically
to enhance excitability. Groups II (mGluR2 and 3) and III
(mGluR4, 6, 7, and 8), in contrast, are primarily located
presynaptically, are negatively coupled to adenylyl cyclase via
Gi/Go, and generally act to limit neurotransmitter release. With
the exception of mGluR6, which is confined to the retina
(Nomura et al. 1994), group II and III mGluRs are distributed
widely throughout the CNS, including the perforant path, the
major excitatory projection to the dentate and hippocampus
(Bradley et al. 1996; Kew et al. 2001; Shigemoto et al. 1997).

To identify specific mGlu receptor subtypes involved in the
regulation of perforant path input, we utilized genetically
manipulated mice (mGluR8 KO and mGluR4/8 double KO
animals) in conjunction with selective group II and III mGluR
pharmacological agonists. We show that presynaptic inhibition
mediated by mGluR7, but not mGluR2/3 or mGluR8, is re-
duced in the MPP of the dentate after PILO-induced SE in
mice.

METHODS

Animals

All experiments were performed in accordance with National In-
stitutes of Health guidelines for the care and use of laboratory animals
and approved by Emory University's Institutional Animal Care and
Use Committee. Metabotropic glutamate receptor 8 $(-/-)$ and
mGluR4 $(-/-)$ animals were obtained as a gift from R. Duvoisin

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(Duvoisin et al. 1995) and P. J. Conn. Colonies of mGluR4/8 double KO were generated by crossing the mGluR4- and mGluR8-deficient strains. Double KOs did not display overt behavioral differences from WT mice, because animals remained active and well groomed for the duration of our study. The mGluR4/8 double KO mice were fertile, with litter sizes within the normal range, and they grew at a normal rate. Furthermore, both groups of mice responded similarly to PILO, suggesting that seizure susceptibility for the mGluR4/8 KO mice was not markedly enhanced over WT controls. WT 129sv1/svImJ mice were purchased from (Jackson Laboratories, Bar Harbor, ME). Male mice, 45–75 days old, were used for all experiments. Targeted disruption of the mGluR4 and 8 genes in KOs was confirmed via PCR. DNA was collected from tail snips (0.5 cm) and isolated using QuickExtract DNA extraction solution (Epicenter, Madison WI). PCR products were separated on a 1.5% agarose gel and visualized with ethidium bromide.

PILO treatment

Animals were injected with atropine methyl bromide (4 mg/kg, ip) (Kobayashi et al. 2003) and, 30 min later, with pilocarpine hydrochloride (320–360 mg/kg, ip). Age-matched controls were injected with an equivalent amount of atropine methyl bromide and 0.9% saline solution. SE was allowed to proceed unabated, typically lasting 1.5–3 h in duration. Only mice that exhibited SE for ≥1.5 h were used for electrophysiological experiments. For the first 72 h following SE, recovering animals were monitored two to four times per day and injected with 0.2–0.4 ml of 5% dextrose in lactated Ringer's solution (ip) as needed, because some of the animals did not eat or drink normally for 1–3 days after SE. As judged by casual observation, at least 2 of 11 animals not used for electrophysiological experiments developed spontaneous seizures after >45 days.

Electrophysiology

Animals were deeply anesthetized with halothane and killed by decapitation. Brains were rapidly removed and chilled in an ice-cold, carbogenated (i.e., bubbled with 95% O_2-5% CO_2) cutting artificial cerebrospinal fluid (ACSF) containing the following (in mM): 130 NaCl, 3.5 KCl, 1.25 Na_2HPO_4, 24 $NaHCO_3$, 4 $MgSO_4$, 1 $CaCl_2$, and 10 glucose (osmolarity 300 ± 5 mOsm). Transverse entorhinal-hippocampal slices (450–500 μM thick) were cut horizontally using a Vibrotome and incubated with cutting ACSF for ~30 min at 25°C. Slices were subsequently transferred to a holding chamber in bathing ACSF containing 2 mM each of $MgSO_4$ and $CaCl_2$, where they were maintained for ≥30 min at room temperature until experiments were begun. Slices were individually transferred to a submerged chamber for recording and continuously perfused with oxygenated (95% O_2-5% CO_2) bathing medium (3–5 ml/min). All experiments were performed at a temperature of 29.5–31°C.

Extracellular field excitatory postsynaptic potentials (fEPSPs) were recorded using glass micropipettes (5–10 MΩ) filled with normal ACSF bathing medium. Slices from the middle hippocampus were used preferentially. Responses were evoked by stimulation (0.1-ms duration, 25–150 μA amplitude) delivered to either the outer molecular layer [lateral perforant path (LPP)] or middle molecular layer [medial perforant path (MPP)] using Teflon-coated, Pt/Ir monopolar microelectrodes (0.9–1 MΩ impedance; Fig. 1A). Stimulus intensity was adjusted to produce 50–75% of the maximal fEPSP amplitude. (S)-3,4-dicarboxyphenylglycine (DCPG), L-(+)-2-amino-4-phosphonobutyric acid (L-AP4), and (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV) were purchased from Tocris Cookson (Ellisville, MO) and bath-applied in all experiments for 10 min. Bicuculline (BIC, 30 μM; Tocris) and D-2-amino-5-phosphonovalerate (D-APV, 30–100 μM; Tocris) were co-applied in most experiments to block GABA$_A$- and N-methyl-D-aspartate (NMDA)-mediated neurotransmission, respectively. All drugs were dissolved in 18.2 MΩ water
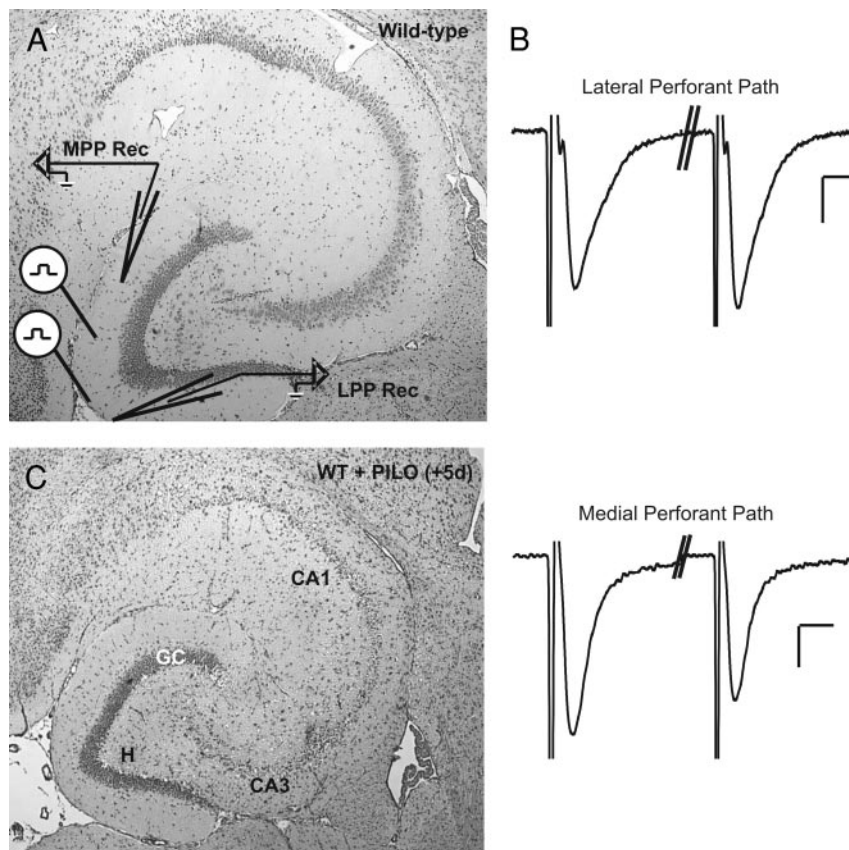

FIG. 1. Pilocarpine (PILO)-induced status epilepticus produced characteristic patterns of neuronal cell loss in mice typical of rat models and human epilepsy. *A*: representative transverse hippocampal slice taken from a saline-injected wild-type (WT) control animal fixed and stained with hematoxylin after recording. Also shown are the characteristic placement of stimulating and recording electrodes in the medial perforant path (MPP) and lateral perforant path (LPP). MPP and LPP responses were evoked by placing the stimulating and recording electrodes into the middle or outer molecular layers, respectively. *B*: electrodes were correctly positioned if paired-pulse stimulation (150-ms interpulse interval) produced depression in the MPP, and facilitation in the LPP. Calibration bars = 5 ms, 0.3 mV. *C*: granule cells (GC) in the dentate gyrus were spared, whereas extensive cell loss in the hilus (H) and areas CA3–CA1 of the hippocampus were typically observed in sections taken from PILO-treated animals (cf. *A* vs. *C*). These data, along with casual observation of spontaneous seizures in at least 2 of 11 animals not used for electrophysiology, suggest that our mice developed epilepsy after PILO.

as 10–60 mM stocks solutions, and if not used immediately, were stored in frozen aliquots for <1 mo. Electrode placements in the MPP and LPP were corroborated by observing paired-pulse depression or facilitation, respectively, at the 150-ms interpulse interval (Fig. 1*B*) (McNaughton 1980) and differential pharmacological responses to the application of L-AP4 or DCPG (Macek et al. 1996; Zhai et al. 2002). Responses that did not exhibit consistent paired-pulse depression (MPP recordings) or facilitation (LPP recordings) were not used.

Histology

PILO-treated rats (Buckmaster and Jongen-Relo 1999; Williams et al. 2002) and mice (Borges et al. 2003; Shibley and Smith 2002) exhibit distinct patterns of neuronal cell loss in the hippocampus after SE, which resembles the pattern of hippocampal cell damage exhibited in patients with temporal lobe epilepsy (TLE) (Bender et al. 2003). To determine if PILO treatment produced similar patterns of neuronal cell loss in the 129svJ mouse strain used in this study, we examined qualitatively the neuronal cell densities throughout the hippocampus and dentate gyrus in control ($n = 5$ mice) and PILO-treated animals ($n = 5$ mice). After recording, slices were fixed in 4% paraformaldehyde, paraffin embedded, sectioned (8 μM), and stained with hematoxylin (Biomedia, Foster City, CA) for light microscopy observation. All sections taken from SE-experienced mice had fewer cells in the hilus and throughout stratum pyramidale (hippocampal areas CA3–CA1), whereas dentate granule cells (GCs) were relatively spared from SE-induced cell death (Fig. 1*C*). Sections made from control mice never displayed these patterns of neuronal cell loss. These data indicate that PILO-induced SE in our mice produced patterns of neuronal cell loss that were characteristic of animal models of and human temporal lobe epilepsy.

Data analysis

The slope of the fEPSP between 20 and 80% of its amplitude was measured by linear regression analysis (Clampfit 9.0, Axon Instruments, Union City, CA). Reductions in the fEPSP slope after application of mGluR agonists (i.e., maximal percent inhibition) were calculated by comparing the mean fEPSP slopes before and after drug application. Baseline measurements were averaged from data 5 min before drug application, whereas agonist-induced inhibition was determined from the average of responses measured 2.5–15 min after drug exposure; this helped to correct for slight variations in wash-in time between low (DCG-IV) and high (L-AP4) concentrations of mGluR agonists. Differences in agonist-induced inhibition between SE-experienced and control groups were compared by ANOVA or two-tailed, unpaired *t*-test, as appropriate. Time course data were subjected to repeated measures ANOVA (GraphPad Prism 4.0, GraphPad Software, San Diego, CA). Differences were considered significant if $P < 0.05$.

RESULTS

mGluR7-mediated inhibition in the MPP is diminished 3–9 days after PILO-induced SE

Although mGluR agonists can differentially activate receptors between groups, most compounds do not discriminate for specific receptor subtypes within groups. To study the mGlu7 receptor subtype in mouse hippocampal slices, we measured the actions of the selective group III agonist, L-AP4 (600 μM), in mice bearing deletions of mGluRs 4 and 8, the other group III mGluRs localized to the dentate (Bradley et al. 1996; Shigemoto et al. 1997). In the MPP of SE-naïve animals, L-AP4 reversibly reduced the fEPSP slope equally in slices taken from either WT or mGluR4/8 double KO mice (Fig. 2,

A–C). In the LPP of untreated animals, L-AP4 (600 μM) suppressed fEPSPs by 40 ± 3% in slices taken from WT mice, however, L-AP4 only slightly inhibited responses in mGluR4/8 double KO animals (5 ± 2%; Fig. 2*D*). This suggests that mGluR7-mediated presynaptic inhibition is functionally limited to the MPP, but not the LPP.

We also asked whether presynaptic inhibition mediated by mGluR7 might be altered after PILO-induced SE. In WT mice, MPP inhibition produced by 600 μM L-AP4 was significantly reduced 3–9 days after PILO-induced SE, changing from 14 ± 3% in controls to 4 ± 2% after PILO (Fig. 2*B*, *left*; $P < 0.05$, unpaired *t*-test). To confirm this result, we performed the same set of experiments using mGluR4/8 double KO animals. As with WT mice, 600 μM L-AP4 was significantly less effective in reducing the MPP fEPSP in PILO-treated mGluR4/8 animals (Fig. 2*B*, *right*; $P < 0.05$, unpaired *t*-test). In the LPP, there were no differences in L-AP4 sensitivity after SE (Fig. 2*D*). These results suggest that presynaptic inhibition mediated by mGluR7 in the MPP is functionally decreased after PILO-induced SE.

Perforant path inhibition mediated by mGluR2/3 is unchanged 3–9 days after PILO-induced SE

mGluR2/3 has been predominantly localized to the MPP, although it has also been described in low levels in the LPP (Shigemoto et al. 1997). In the MPP of untreated animals, the selective group II agonist, DCG-IV (300 nM), reversibly depressed the slope of the fEPSP by 55 ± 2% in WT and 58 ± 4% in mGluR4/8 double KO animals (Fig. 3, *A–C*). In the LPP, DCG-IV (300 nM) depressed the slope of the fEPSP by 26 ± 3% in WT animals and 13 ± 5% in mGluR4/8 KO mice.

Group II mGluR function is enhanced in the central amygdala after electrical kindling (Neugebauer et al. 2000) and in the hilus after PILO-induced SE (Doherty and Dingledine 2001). In this study, group II mGluR-mediated inhibition in the MPP was similar in both WT and mGluR4/8 double KO animals 3–9 days after PILO-induced SE (Fig. 3, *A–C*). In the LPP, the peak level of inhibition exhibited also did not change after SE for either WT or mGluR4/8 double KO mice after PILO (Fig. 3*D*). This suggests that presynaptic inhibition mediated by mGluR2/3 in the perforant path is not reduced 3–9 days after PILO-induced SE, and accordingly, does not likely contribute to epileptogenesis.

Group III mGluR presynaptic inhibition in the LPP is mediated exclusively by mGluR8 and is not diminished 3–9 days after PILO-induced SE

DCPG appears to be a highly selective mGluR8 activator (Thomas et al. 2001). To evaluate the selectivity of DCPG in mouse hippocampal slices, we measured its actions in the LPP of WT animals and mice bearing deletions of mGluR8. We also applied the well-described group III agonist, L-AP4, in a concentration known to selectively activate mGluR4 and 8, but not mGluR7 (60 μM), in heterologous receptor-expression systems (Conn and Pin 1997) and compared its actions to those responses elicited by the application of DCPG.

Immunohistochemically, mGluR8 appears to be located exclusively in the LPP, expressed within the outer one-third of the molecular layer (Shigemoto et al. 1997; Zhai et al. 2002).
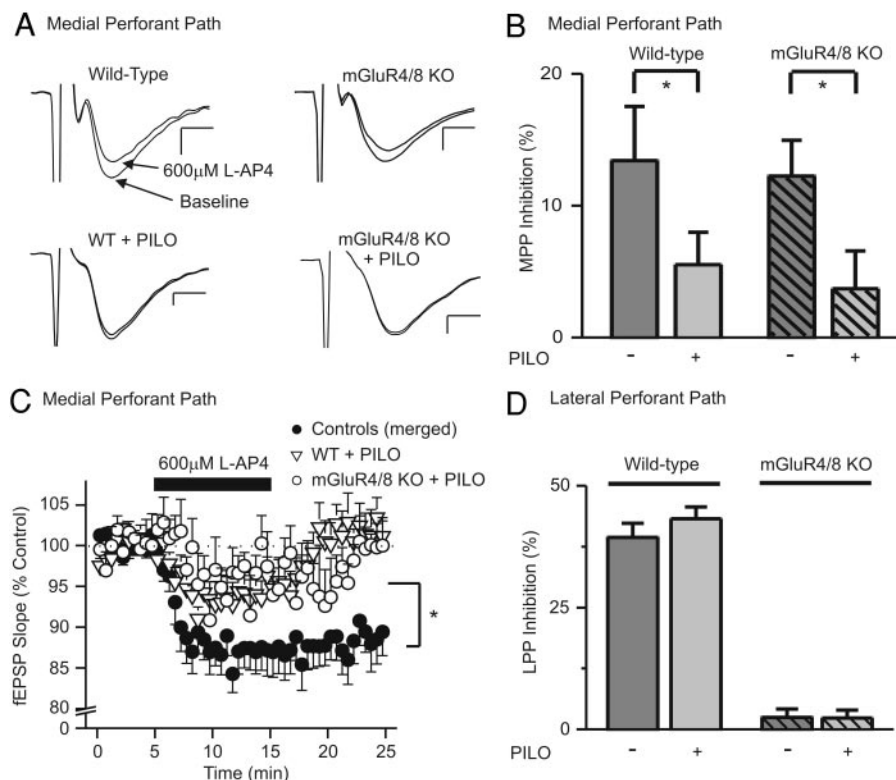
FIG. 2. Synaptic inhibition mediated by metabotropic glutamate receptor (mGluR)7 after PILO-induced status epilepticus. *A*: sample traces from a WT control and PILO-treated, mGluR4/8 knockout (KO) control and mGluR4/8 PILO-treated mice. Depicted field excitatory postsynaptic potentials (fEPSPs) were characteristic of the level of inhibition observed for controls prior to (baseline) and in the presence of the selective group III mGluR agonist, L-(+)-2-amino-4-phosphonobutyric acid (L-AP4, 600 μM), in the MPP. Calibration bars = 2 ms, 0.3 mV. *B*: mean level of inhibition produced by L-AP4 (600 μM) in the MPP was significantly reduced after PILO-induced SE in both WT ($P < 0.05$, unpaired *t*-test) and mGluR4/8 double KO mice ($P < 0.05$, unpaired *t*-test). Bars represent group means for WT control ($n = 9$ slices taken from 6 mice), WT PILO-treated mice ($n = 9$ slices, 5 mice), mGluR4/8 KO control ($n = 9$ slices, 6 mice), and mGluR4/8 double KO PILO-treated animals ($n = 7$ slices, 4 mice) $\pm$ SE. *C*: time course averages for experiments during the application of L-AP4 in the MPP. Since both untreated WT and mGluR4/8-KO mice were not significantly different from one another, WT and mGluR4/8 KO controls were averaged and graphically shown together (●); the WT PILO- (○) and mGluR4/8 PILO-treated (▽) are also shown. All points are means $\pm$ SE. *$P < 0.001$, repeated measures ANOVA with post hoc Bonferroni's multiple comparison test. *D*: peak level of inhibition produced by the selective group III agonist in the LPP for WT and mGluR4/8 double KO mice. WT controls: $n = 10$ slices, 6 mice; PILO-treated WT animals: $n = 10$ slices, 5 mice; mGluR4/8 KO controls: $n = 8$ slices, 5 mice; mGluR4/8 KO PILO-treated animals: $n = 10$ slices, 4 mice. Bars represent group means $\pm$ SE.

In slices from untreated WT animals, the dose-response curve for DCPG yielded a median effective concentration (EC$_{50}$) in the LPP of 900 nM (Fig. 4*A*). To look for reductions in inhibition, a maximal concentration of DCPG (3 μM) was applied. At 3 μM, DCPG reversibly inhibited evoked fEPSPs in the LPP of SE-naïve animals by 26 $\pm$ 3%, but was without effect (0 $\pm$ 2%) in slices taken from untreated mGluR8 KO mice (Fig. 4, *A–D*). L-AP4, by comparison, produced a peak inhibition of 19 $\pm$ 2% in the LPP, and did not affect (3 $\pm$ 2%) slices taken from mGluR8 KO animals (Fig. 4*D*). In the MPP, DCPG slightly inhibited WT mice (6 $\pm$ 1%) and was without effect in mGluR8 KO animals (0 $\pm$ 1%; Fig. 4, *E* and *F*). This finding suggests that mGluR8 alone mediates presynaptic inhibition in the LPP.

Previous studies have shown that mGluR8 function in the perforant path is chronically reduced after the development of epilepsy (Dietrich et al. 1999; Kral et al. 2003; Tang et al. 2001), suggesting a reduction of presynaptic inhibition of glutamate release may contribute to maintained hyperexcitability within the dentate. To determine if mGluR8-mediated presynaptic inhibition is also reduced before spontaneous sei-

zures begin (i.e., during epileptogenesis), the sensitivity to DCPG within the LPP was measured during the latent period in mice. Three to 9 days after SE in PILO-treated WT mice, DCPG-mediated inhibition was unchanged in the LPP (Fig. 4, *B–D*). The peak level of inhibition was 23 $\pm$ 2% for PILO-treated animals versus 26 $\pm$ 3% for WT controls. Similarly, DCPG-induced inhibition in the MPP was not altered after PILO (6 $\pm$ 1% inhibition in control vs. 8 $\pm$ 2% after SE; Fig. 4, *E* and *F*). These results indicate mGluR8-mediated presynaptic inhibition is not reduced 3–9 days after PILO-induced SE and suggest that the late reduction in mGluR8 function observed in spontaneously seizing animals (Dietrich et al. 1999; Kral et al. 2003; Tang et al. 2001) might be a consequence of spontaneous recurrent seizures.

DISCUSSION

The main conclusions of this study are the following. First, in the LPP of untreated animals, presynaptic inhibition is mediated by mGluR2/3 and 8, but not mGluR4 or 7. Second, in
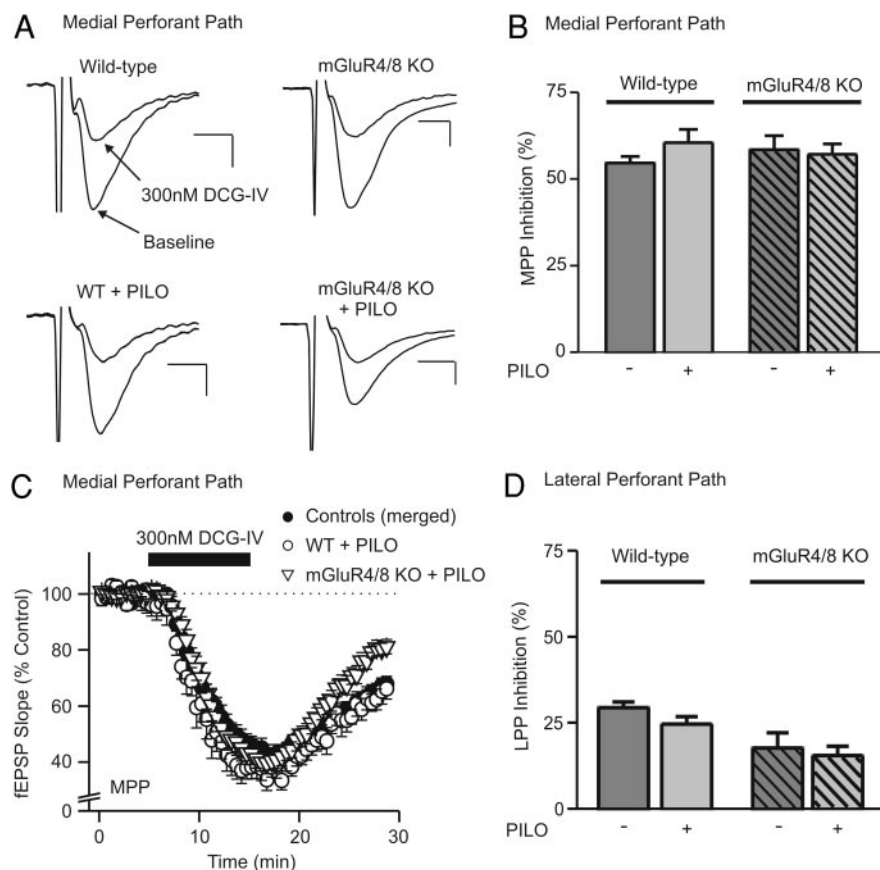
FIG. 3. Synaptic inhibition mediated by mGluR2/3 in the perforant path is not altered 3–9 days after PILO-induced status epilepticus. *A*: representative fEPSPs showing the selective mGluR2/3 agonist, (2′*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl) glycine (DCG-IV, 300 nM) reduced responses in the MPP of WT control and PILO-treated animals and mGluR4/8 double KO control and PILO-treated mice to a similar degree. Calibration bars = 3 ms, 0.3 mV. *B*: summary bar graph showing the peak levels of inhibition in the MPP was not changed after PILO-induced SE in either WT or mGluR4/8 KO mice. Error bars are ±SE. *C*: time course for DCG-IV-mediated inhibition (300 nM, bar) in the MPP of the dentate gyrus. Since non–PILO-treated WT ($n = 10$ slices taken from 6 mice) and mGluR4/8-KO ($n = 9$ slices, 5 mice) control mice were not significantly different from one another, these groups were averaged and graphically shown together (●). WT PILO-treated mice (○, $n = 9$ slices, 5 animals) and the mGluR4/8 double KO PILO-treated animals (▽, $n = 9$ slices, 4 mice) are also shown. Symbols represent means ± SE. Agonist was bath applied for 10 min (bar). *D*: mean data showing the peak level of fEPSP inhibition attained after DCG-IV (300 nM) in the LPP. WT controls: $n = 10$ slices, 6 mice; PILO-treated WT animals: $n = 9$ slices, 5 mice; mGluR4/8 KO controls: $n = 7$ slices, 5 mice; mGluR4/8 KO PILO-treated animals: $n = 9$ slices, 4 mice. Bars are group means ± SE.

the MPP of untreated animals, synaptic transmission is inhibited by activation of mGluR2/3 and 7, but not mGluR4 or 8. Third, after PILO-induced SE, mGluR7-mediated inhibition is significantly diminished in the MPP, whereas mGluR8 (LPP) and mGluR2/3 (MPP and LPP)-mediated inhibition are not altered. These findings indicate that presynaptic group II/III mGluR-mediated inhibition within the perforant path of the dentate is remarkably layer-specific and is consistent with the notion that a reduction of mGluR7 function may contribute to epileptogenesis.

Several observations indicate that mGluR7 is the only group III mGluR to mediate presynaptic inhibition within the MPP. First, the high concentration of L-AP4 (600 μM) required to inhibit fEPSPs (Fig. 2, *A–C*) suggests that the mGlu7 receptor subtype mediated this response (Conn and Pin 1997; Wu et al. 1998). Second, the same concentration of L-AP4 produced a level of inhibition in mGluR4/8 double KO mice that was equivalent to WT mice (Fig. 2, *A–C*). Third, because a lower concentration of L-AP4 (60 μM) is likely to activate only mGluR4 and mGluR8, the lack of an effect of L-AP4 in untreated WT mice (Fig. 4*F*) further indicates that only mGluR7 inhibits presynaptic glutamate release in the MPP.

mGluR7-mediated inhibition in the MPP was reduced 3–9 days after SE (Fig. 2, *A–C*). Because there is extensive cell loss in the entorhinal cortex in animal models (Kobayashi et al. 2003) or human epilepsy (Duvoisin et al. 1995), it might be expected that the loss of mGluR7-bearing afferent projections might account for the diminished L-AP4 mediated inhibition within the MPP we observed. Indeed, mGluR7b-bearing hilar mossy cells, which project to the inner molecular layer of the

dentate gyrus, have been shown to be preferentially lost in epileptic patients with Ammon's Horn Sclerosis (Blumcke et al. 2000). However, it is the neurons found in medial portion of layer II that comprise the afferents of the MPP (Witter et al. 2000), and the majority of cell loss in the entorhinal cortex occurs in layer III after PILO (Kobayashi et al. 2003). Thus it seems improbable that a selective loss in mGluR7-bearing neurons explains these data. Rather, this finding suggests that a down-regulation or ineffectual downstream second messenger coupling is more likely to account for the observed reduction in mGluR7-mediated presynaptic inhibition.

Activation of mGluR2/3 by DCG-IV (300 nM) in the MPP diminished the slope of the fEPSP of both WT and mGluR4/8 double KO animals equally (Fig. 3, *A* and *B*), similar to levels described previously for rats (Kew et al. 2001; Macek et al. 1996) and mice (Kew et al. 2002). DCG-IV also inhibited evoked responses in the LPP (Fig. 3*D*), where the level of inhibition in the LPP (25–30%) was approximately one-half that observed in the MPP (55–60%). These results are consistent with immunohistochemical (Shigemoto et al. 1997) and electrophysiological (Lovinger and McCool 1995; Macek et al. 1996) data describing a role for mGluR2/3 in the LPP and indicate a potent regulatory role for mGluR2/3 across multiple synapses (i.e., both LPP-to-GC and MPP-to-GC) within the dentate molecular layer.

Previous work has shown that DCG-IV (either 1 μM or 200 nM) produced the same level of inhibition at the perforant path-to-GC synapse in sclerotic hippocampal tissue resected from epileptic patients as it did in nonsclerotic control tissue (Dietrich et al. 2002). In rats, mGluR2-mediated synaptic
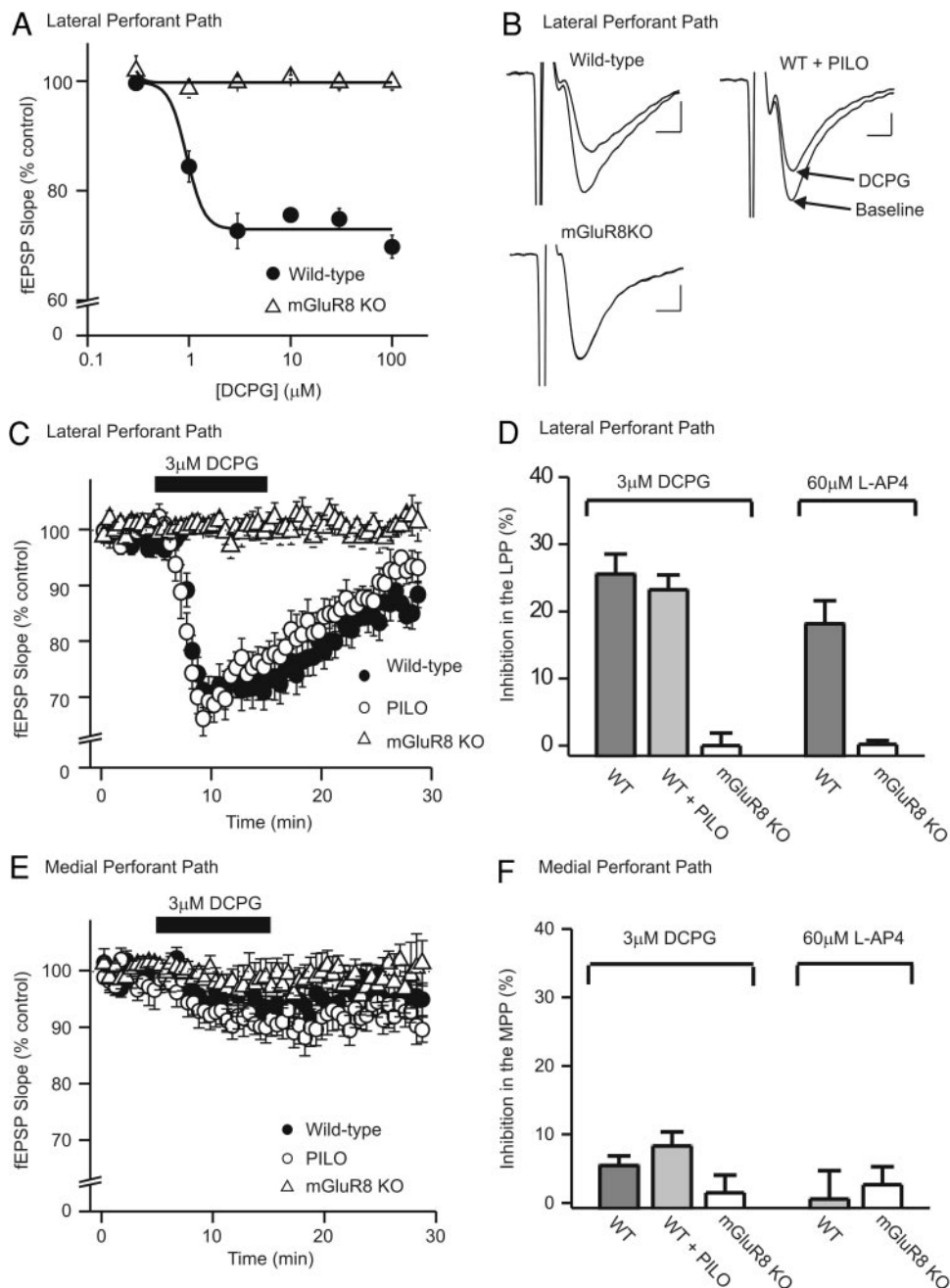
FIG. 4. Synaptic inhibition by (S)-3,4-dicarboxyphenylglycine (DCPG, 3 μM), a highly selective mGluR8 agonist, in the (A–D) LPP and (E and F) MPP of the dentate gyrus after PILO-induced status epilepticus. A: dose-response curve for DCPG in the LPP. DCPG inhibits responses evoked in the LPP of WT (non–PILO-treated) slices, but not in slices taken from mGluR8 KO animals. B: representative fEPSPs showing DCPG (3 μM) inhibited responses in the LPP of WT control and PILO-treated animals equally, but did not inhibit LPP responses in slices taken from mGluR8-KO mice. Calibration bars = 2 ms, 0.3 mV. C: time course for DCPG-induced effects in the LPP for WT controls (●, n = 10 slices from 7 mice), PILO-treated (○, n = 9 slices, 5 mice), or mGluR8 KO (△, n = 8 slices, 6 mice). Symbols represent means ± SE. Horizontal bars indicate duration of drug application. D: summary data showing peak levels of inhibition in the LPP after the application of DCPG compared with the selective group III agonist, L-AP4 (60 μM; a concentration to activate mGluR4 + mGluR8, but not mGluR7; see RESULTS). Numbers of animals summarized here for 3 μM DCPG in the LPP were as described in C. Numbers of animals used in experiments involving 60 μM L-AP4 were as follows: WT controls, n = 5 slices, 5 mice; mGluR8 KO, n = 4 slices, 3 mice; mGluR8 KO, n = 4 slices, 4 mice. Bars represent means ± SE. E: time course for these effects in the MPP for WT controls (●, n = 11 slices, 7 mice), PILO-treated (○, n = 8 slices, 5 mice), or mGluR8 KO (△, n = 8 slices, 6 mice). F: summary of peak levels of inhibition in the MPP after either DCPG (3 μM) or L-AP4 (60 μM). Numbers of animals summarized here for 3 μM DCPG in the MPP were as described in E. Numbers of animals used in experiments involving 60 μM L-AP4 were as follows: WT controls, n = 5 slices, 5 mice; mGluR8 KO, n = 4 slices, 4 mice. Bars represent means ± SE.

plasticity was not altered at perforant path inputs to hilar-border interneurons after PILO-induced SE (Doherty and Dingledine 2001). In this study, inhibition mediated by mGluR2/3 was unchanged 3–9 days after PILO-induced SE in both the MPP (Fig. 3, A–C) and the LPP (Fig. 3D). These data indicate that DCG-IV was equally effective at inhibiting the MPP and LPP regardless of PILO treatment, and collectively suggest that the mGluR2/3 receptor subtypes in the perforant path do not contribute to the early development of epilepsy.

The selective inhibitory effects of DCPG within the LPP (Fig. 4, A–D) confirm a role for mGluR8-mediated inhibition in the perforant path (Zhai et al. 2002) and suggest that this presynaptic inhibition is confined to the LPP. It is unlikely that inhibition in the LPP is mediated by mGluR4, because 60 μM L-AP4 produced the same level of inhibition as did 3 μM

DCPG in WT mice and did not produce any inhibition in mGluR8 KO mice (Fig. 4D).

Mice did not exhibit any significant alteration in DCPG-mediated inhibition after PILO-induced SE (Fig. 4, B–D). This is internally consistent with our results after the application of the other group III agonist used here, L-AP4, showing a lack a change after SE in the LPP (Figs. 2D and 4D). In light of previous findings describing diminished group III–mediated perforant path inhibition at the same LPP-to-GC synapse after SE (Dietrich et al. 1999; Klapstein et al. 1999; Kral et al. 2003), the lack of reduced mGluR8-mediated inhibition after PILO in this study was somewhat surprising. One possible explanation for these results may be that mGluR8 is decreased in the chronic state, but not during the latent period. We measured DCPG responses during the latent period (i.e., 3–9

days after SE), whereas others investigated mGluR8-mediated inhibition after >30 days after SE (Dietrich et al. 1999; Klapstein et al. 1999; Kral et al. 2003). Tang et al. (2001) reported that mGluR8 immunoreactivity was up-regulated 1 day after PILO in the entire molecular layer, returned to control values by 3 days after PILO, and progressively diminished for the next 28 days until it was almost completely lost by 31 days after SE. Thus, because we measured mGluR8-mediated inhibition in the LPP 3–9 days after PILO, it is possible that a functional reduction had yet to occur. Our data, along with the aforementioned studies, indicate that if mGluR8 is involved in the development of epilepsy, its role is not apparent 3–9 days after PILO-induced SE. However, a chronic reduction in mGluR8-mediated inhibition (Dietrich et al. 1999; Kral et al. 2003; Tang et al. 2001) may be important for maintenance of the epileptic phenotype.

Implications of diminished mGluR7-mediated inhibition in epilepsy

The diminution of mGluR7-mediated inhibition in the MPP noted here after SE (Fig. 2) is consistent with a possible role for aberrant excitation within the dentate. On the other hand, the magnitude of mGluR7-mediated inhibition in the MPP is not large, even in untreated animals (Fig. 2, *B* and *C*). However, how might this reduction in mGluR7 contribute to the development of hippocampal hyperexcitability and/or spontaneous seizures? Several lines of evidence suggest that changes in mGluR7 are likely to play an integral part in regulating the balance between excitation and inhibition. First, group III agonists suppress epileptiform activity when applied in vitro (Burke and Hablitz 1994) or perfused locally in vivo (Abdul-Ghani et al. 1997; Folbergrova et al. 2003). Second, mGluR7

is apparently required for long-term depression (LTD) of excitatory inputs to rat CA3 stratum radiatum interneurons (Laezza et al. 1999). Alterations in synaptic plasticity can powerfully refine synaptic connectivity and have long been thought to importantly contribute to hyperexcitability and epilepsy (Schwartzkroin 2001). Third, a focal injection of the group III agonist L-SOP into the inferior colliculus produced a short (10 min) proconvulsant excitation in sound-sensitive genetically epilepsy-prone rats, followed by a prolonged phase of enhanced protection from subsequent sound-induced seizures that lasted for 2–4 days (Yip et al. 2001). The authors reported an up-regulation in both mRNA and protein levels of mGluR7 in the inferior colliculus that they hypothesized was responsible for the prolonged anticonvulsant effect. Finally, it is important to note that only mice lacking mGluR7 display an increased susceptibility to seizures, whereas other mGluR1, 2, 4, 5, and 8 KO mice do not (Linden et al. 2002; Sansig et al. 2001; Snead et al. 2000). Sansig et al. (2001) attributed the enhanced seizure susceptibility exhibited by mGluR7-deficient mice in part to "very small excitability changes" in hippocampal area CA1 and deficits in recovery from activity-dependent facilitation in neocortex.

Our working hypothesis to describe how the observed changes in mGluR7 function might contribute to dentate hyperexcitability is depicted in Fig. 5. In the latent period after SE, the observed reduction in mGluR7-mediated presynaptic inhibition in the MPP (Fig. 2) would be expected to enhance, perhaps in a subtle manner, the response of GCs to input trains from entorhinal cortex. At the same time, increased mGluR2/3-mediated presynaptic inhibition of GC inputs to hilar-border interneurons (Doherty and Dingledine 2001) would be expected to partially disinhibit GCs, further increasing their firing
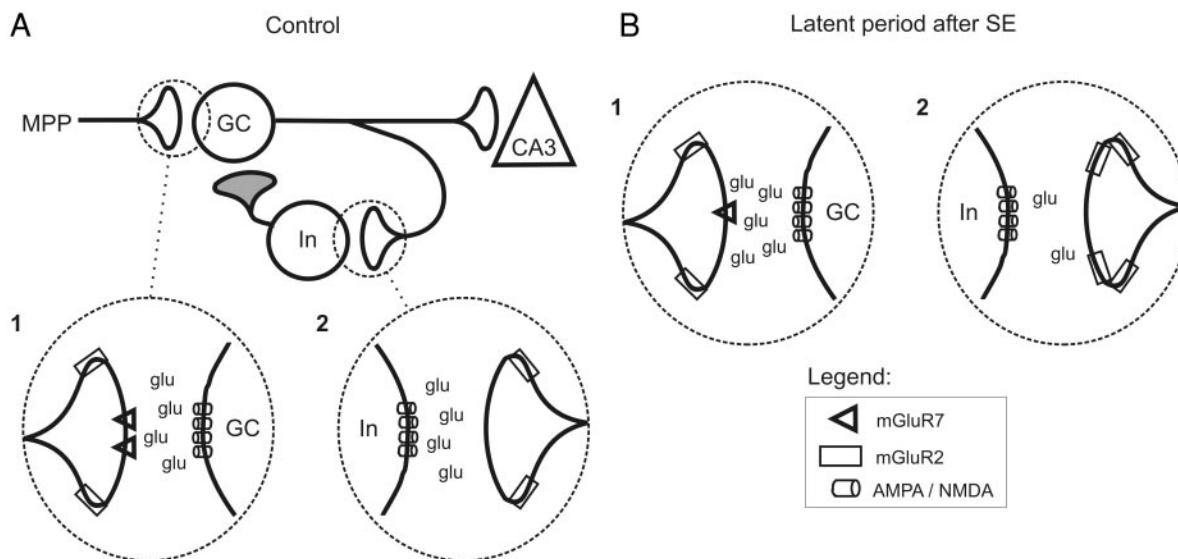

FIG. 5. Diagram showing how the present mGluR-mediated changes might contribute to increased neuronal transfer through the dentate and epileptogenesis. *A*: under normal conditions, neuronal transfer through dentate granule cells (GCs) is tightly regulated. Input to GCs is limited by presynaptic inhibition mediated by mGluR2/3 and 7 (*1*) and output is controlled in part by feedback inhibition via GABAergic interneurons (In) (*2*). *B*: however, during the latent period 3–9 days after PILO-induced SE, attenuated presynaptic inhibition mediated by mGluR7 in the MPP would allow for enhanced glutamate release and subsequent hyperexcitation of GCs (*1*). This would be expected to act synergistically with increased mGluR2/3-mediated depression of excitatory drive of hilar-border interneurons (Doherty and Dingledine 2001) (*2*), which would be expected to reduce feedback inhibition. These combined effects mediated by presynaptic mGluRs—enhanced excitatory input of dentate GCs in conjunction with diminished feedback inhibition—might produce and/or enhance hyperexcitability within the dentate circuit and contribute to the development of spontaneous seizures.

probability during a train of inputs. The combination of enhanced excitatory input and reduced feedback inhibitory control of GCs—both achieved by alterations in mGluR-mediated presynaptic regulation of glutamate release—are proposed to act synergistically to increase the propagation of neuronal signals through the dentate into CA3 during the epileptogenic period. This hypothesis is consistent with findings of enhanced MPP input to, and reduced inhibition of, dentate GCs 3–7 days after PILO-induced SE (Kobayashi et al. 2003). Investigations of enhanced dentate throughput are underway to further test this hypothesis.

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