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Metabotropic glutamate receptor signaling is required for NMDA receptor-dependent ocular dominance plasticity and LTD in visual cortex

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A feature of early postnatal neocortical development is a transient peak in signaling via metabotropic glutamate receptor 5 (mGluR5). In visual cortex, this change coincides with increased sensitivity of excitatory synapses to monocular deprivation (MD). However, loss of visual responsiveness after MD occurs via mechanisms revealed by the study of long-term depression (LTD) of synaptic transmission, which in layer 4 is induced by acute activation of NMDA receptors (NMDARs) rather than mGluR5. Here we report that chronic postnatal down-regulation of mGluR5 signaling produces coordinated impairments in both NMDAR-dependent LTD in vitro and ocular dominance plasticity in vivo. The data suggest that ongoing mGluR5 signaling during a critical period of postnatal development establishes the biochemical conditions that are permissive for activity-dependent sculpting of excitatory synapses via the mechanism of NMDAR-dependent LTD.

Results

Chronic Inhibition of mGluR5 Signaling Impairs Ocular Dominance Plasticity. Our experiments were motivated by the finding of impaired ocular dominance plasticity in Gmis5+/− mice (Fig. 1 A–C). This finding was surprising on two counts. First, other thanocular compounds (20–22); however, subsequent experiments did confirm that NMDAR-dependent LTD occurs normally in layers 2/3 of visual cortex in Grm5 knockout mice (23).

The idea that mGluR5 is critically involved in visual cortical plasticity in vivo was rekindled with the finding that deprived-eye depression fails to occur in layer 4 of Grm5−/− mutant mice (24). This finding was unexpected because, as reviewed above, a considerable body of evidence has implicated the mechanism of NMDAR-dependent LTD in deprived-eye depression. In the present study, we reexamined the role of mGluR5 in LTD and ocular dominance plasticity in layer 4, using the Grm5−/− mouse and a highly specific negative allosteric modulator, 2-chloro-4-[(2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl]pyridine (CTEP), that has proven suitable for chronic inhibition of mGluR5 (25, 26). Our data show that NMDAR-dependent LTD and deprived-eye depression in layer 4 require mGluR5 signaling during postnatal development.

Significance

Interruption of normal sensory experience during early postnatal life often causes a permanent loss of synaptic strength in the brain and consequent functional impairment. For example, temporary monocular deprivation causes long-term depression (LTD) of synapses in the visual cortex of mammals, along with a profound loss of vision. The mechanisms by which this synaptic plasticity occurs are only partially understood. Here we show that signaling via metabotropic glutamate receptor 5 during a critical period of postnatal development establishes the biochemical conditions that are permissive for activity-dependent sculpting of excitatory synapses via the mechanism of NMDA receptor-dependent LTD.

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dominance plasticity, broad phenotypic screens had shown little consequences of knocking down mGluR5 by 50% compared with wild type (WT) (24, 27). Second, the deficit in deprived-eye depression in layer 4 after 3 d of MD was reminiscent of the effects of inhibiting NMDAR-dependent LTD (e.g., refs. 10 and 11), which was believed to be unaffected by mGluR5 blockade (23). Therefore, we set out to reexamine the role of mGluRs in ocular dominance plasticity using a different method of mGluR5 inhibition. CTEP is a highly selective mGluR5 negative allosteric modulator that can achieve a steady-state ~75% receptor occupancy in mice by dosing 2 mg/kg s.c. every second day (25, 26). Mice were administered CTEP beginning at postnatal day (P) 21 and continuing throughout the duration of the 3-d MD (Fig. 1D). CTEP had a significant effect on the magnitude of deprived (contralateral) eye depression (P = 0.02, MD x treatment interaction, two-way repeated-measures ANOVA) (Fig. 1 E–H). Both vehicle and CTEP-treated WT mice showed depression of the visual evoked potentials (VEPs) evoked by the contralateral eye (post hoc effect of MD within vehicle, P < 0.001; post hoc effect of MD within CTEP, P = 0.02), but the magnitude of this depression was markedly reduced by CTEP treatment. For VEPs evoked by the ipsilateral eye, there was no interaction between drug treatment and MD (P = 0.264). The fractional change in responses through the ipsilateral and contralateral eyes after MD (Fig. 1G) reveals a significant difference in the ocular dominance shift between treated mice and control mice (P = 0.008; MANOVA). The magnitude of baseline VEPs evoked before MD by the contralateral eye and ipsilateral eye did not differ significantly between vehicle treatment and CTEP treatment (P = 0.255 for contralateral VEPs, P = 0.964 for ipsilateral VEPs, Student t test) (Fig. 1H). These findings, considered together with previous findings in the Grm5+/− mice, indicate that a threshold level of mGluR5 signaling during postnatal development is necessary for ocular dominance plasticity in visual cortex.

Fig. 1. Chronic inhibition of mGluR5 impairs deprived-eye depression in WT mice. (A) Schematic of contralateral and ipsilateral eye inputs to mouse binocular visual cortex. (B and C) WT mice, 3 d of MD induces an ocular dominance shift, expressed primarily as depression of VEP responses driven by the deprived (contralateral) eye. Grm5+/− mice display deficient deprived-eye depression. Data are replotted from Dolen et al. (24). (D) CTEP or vehicle treatment beginning on P21 and lasting throughout the 3-d MD. (E) Averaged waveforms across all experiments, pre- and post-MD. (F) MD-induced depression of the contralateral eye-driven VEP is impaired with CTEP compared with vehicle treated. Data are normalized to day 0 ipsilateral response (vehicle, n = 9; CTEP, n = 14). (G) Average fractional changes in the contralateral eye- and ipsilateral eye-driven VEP responses after MD. CTEP treatment had a significant effect on the magnitude of the ocular dominance shift. (H) Raw VEP amplitudes pre- and post-MD plotted by animal. Vehicle contralateral VEP: pre-MD, 191 ± 17 μV; post-MD, 11 ± 18 μV; CTEP contralateral VEP: pre-MD, 168 ± 11 μV; post-MD, 138 ± 10 μV. Not plotted: vehicle ipsilateral VEP: pre-MD, 86 ± 11 μV; post-MD, 112 ± 12 μV; CTEP ipsilateral VEP: pre-MD, 85 ± 9 μV; post-MD, 92 ± 7. Error bars indicate SEM.
NMDAR-dependent LFS-LTD is impaired in layer 4 with genetic reduction of Grm5.

Grm5 gene dosage nor CTEP treatment affected the intrinsic membrane resistance (Rm) of layer 4 neurons [Rm (Ωm): WT, 94.6 ± 11.1; Grm5−/−, 91.2 ± 21.8; Grm5+/−, 108.9 ± 16.9; vehicle, 108.8 ± 24.7; CTEP, 91.1 ± 13.5].

Given the voltage-dependence of NMDAR conductance, NMDAR-dependent forms of synaptic plasticity are particularly sensitive to levels of inhibition. For example, a genetic reduction in GABAergic inhibition impairs LTD (33) and ocular dominance plasticity (34) in mouse visual cortex. Therefore, we asked whether layer 4 LTD. We found that the LTD was indeed blocked by 50 μM d-(-)-2-amino-5-phosphonopropionic acid (d-APV), an NMDAR antagonist (P = 0.956, pre- and post-LFS, paired Student t test) (Fig. 2D), but not by 60 μM cyclothiazide, a protein synthesis inhibitor that interferes with expression of mGluR5-dependent LTD in the hippocampus (P = 0.014, pre- and post-LFS, paired Student t test) (Fig. 2D). In addition, acute inhibition of mGluR5 with the selective negative allosteric modulator 2-methyl-6-(phenylethynyl)pyridine (MPEP; 10 μM) had no effect on LTD.

Under some experimental conditions, blockade of mGluR-dependent LTD in the hippocampus requires inhibition of both mGluR5 and mGluR1 (25); thus, we also tested whether simultaneous inhibition of both group 1 mGluRs, using MPEP and (S)-(+)-α-amino-4-carboxy-2-methylbenzenacetic acid (LY37385; 100 μM), would inhibit LFS-LTD in layer 4. We found no effect of acute pharmacologic group 1 mGluR inhibition on LTD magnitude (P = 0.939, one-way ANOVA) (Fig. 2E). We also tested whether acute CTEP administration impaired LFS-LTD. Owing to the formulation of CTEP as a microsuspension (25), it was not technically feasible to bath-apply CTEP during slice recordings; therefore, we administered a single dose of CTEP in vivo 3 h before ex vivo slicing and LTD experiments. This drug administration regimen, which is sufficient to impair mGluR-dependent plasticity in CA1 (26), did not affect the magnitude of LFS-LTD in layer 4 (P = 0.886) (Fig. S1).

The effects of chronic and acute inhibition of mGluR5 on LTD are compared in Fig. 2F. These findings indicate that mGluR5 activation is not a trigger for LTD induction in layer 4 of visual cortex, but that mGluR5 signaling during postnatal development is necessary to establish the conditions that make LTD in visual cortex possible.

**NMDAR Function and Inhibition Are Unaffected by Chronic Inhibition of mGlu5.** Because genetic knockdown and chronic pharmacologic inhibition of mGluR5 resulted in impaired NMDAR-dependent plasticity in vivo and in vitro, we tested whether NMDARs are functionally impaired in Grm5 mutants. We first confirmed that basal synaptic transmission, driven mainly by AMPAR-mediated currents, was normal in Grm5−/− and Grm5+/− mice, as measured by input/output (I/O) functions (P = 0.985 for extracellular recordings and P = 0.628 for intracellular recordings, two-way repeated-measures ANOVA, no interactions between stimulation intensity and genotype) (Fig. 3A). Given that basal transmission was normal, we used the AMPA/NMDA ratio as a way to assay NMDAR function. AMPA and mixed AMPA/NMDA-mediared currents were isolated in layer 4 neurons, and showed no difference in Grm5−/− or Grm5+/− mice compared with WT controls (P = 0.990, one-way ANOVA) (Fig. 3B). Western blot analysis of the obligatory NMDAR subunit NR1 also showed no significant differences in WT, Grm5−/−, and Grm5+/− visual cortical slices (P = 0.766, one-way ANOVA) (Fig. 3C). As expected, mGluR5 protein expression was decreased as a function of genotype (P < 0.001, one-way ANOVA) (Fig. 3C).

In both hippocampus and layer 2/3 of visual cortex, there is evidence that mGluR5 is involved in the developmental shift in the NMDAR NR2 subunit from predominantly NR2B to predominantly NR2A (29). Specifically, Grm5−/− mice show enhanced synaptic expression of NR2B during development. The nature of the NR2 subunits regulates the conductance of NMDARs and intracellular protein interactions, and thus their functional consequences when activated (30). The relative levels of NR2A and NR2B in visual cortex are known to have important consequences for the induction of NMDAR-dependent plasticity. NR2A knockout mice display impaired LTD-LTD induced by 1-Hz stimulation and impaired ocular dominance plasticity (9, 31); thus, we hypothesized that mGluR5 regulates plasticity in visual cortex via regulation of the developmental NR2B-to-NR2A shift.

We tested this hypothesis by measuring the decay kinetics of NMDAR-mediated excitatory postsynaptic currents (EPSCs) in layer 4 neurons in slices from animals treated chronically with either CTEP or vehicle. NR2A currents have faster kinetics than NR2B currents (32); however, chronic CTEP treatment did not affect the decay kinetics of layer 4 neurons at P21–P25 (P = 0.940, Student t test) (Fig. 3D). There was also no difference in either the decay kinetics of layer 4 neurons (P = 0.729, one-way ANOVA) (Fig. 3D) or the protein expression of NR2A and 2B subunits in visual cortical slices from Grm5−/− or Grm5+/− mice (P = 0.168 for NR2A, P = 0.434 for NR2B, one-way ANOVA) (Fig. 3C). Neither Grm5 gene dosage nor CTEP treatment affected the intrinsic membrane resistance (Rm) of layer 4 neurons [Rm (Ωm): WT, 94.6 ± 11.1; Grm5−/−, 91.2 ± 21.8; Grm5+/−, 108.9 ± 16.9; vehicle, 108.8 ± 24.7; CTEP, 91.1 ± 13.5].

Given the voltage-dependence of NMDAR conductance, NMDAR-dependent forms of synaptic plasticity are particularly sensitive to levels of inhibition. For example, a genetic reduction in GABAergic inhibition impairs LTD (33) and ocular dominance plasticity (34) in mouse visual cortex. Therefore, we asked whether
inhibition was functionally altered in visual cortex by mGluR5 knockdown. We measured evoked IPSCs and inhibitory postsynaptic currents (IPSCs) within individual layer 4 neurons in response to varying intensities of white matter stimulation (35). We found no significant change in EPSC or IPSC magnitude as a function of Grm5 genotype (main effect of genotype: $P = 0.546$ for EPSCs, $P = 0.464$ for IPSCs, two-way repeated-measures ANOVA) (Fig. 3E).

**NMDAR-Dependent Synaptic Strengthening Persists After Partial, but Not Complete, Inhibition of mGluR5.** We next assessed whether the requirement for mGluR5 signaling was limited to forms of synaptic weakening in layer 4 or was generalized to other forms of NMDAR-dependent plasticity. Stimulus-specific response potentiation (SRP) is an experience-dependent form of synaptic strengthening in visual cortex that requires NMDAR activation and occurs through mechanisms shared with canonical long-term potentiation (LTP) (36, 37). During SRP, repeated exposure to a visual stimulus potentiates VEPs that are evoked by this familiar stimulus, but not by a stimulus of novel orientation (Fig. 4A). We found a significant effect of Grm5 genotype on SRP, as measured by growth of VEP magnitude over days ($P = 0.011$, genotype × day interaction, two-way repeated-measures ANOVA) (Fig. 4B and C). We also found a significant effect of Grm5 genotype on the ability to distinguish between familiar and novel stimuli on day 6 of testing ($P = 0.001$; one-way ANOVA) (Fig. 4D). Post hoc tests revealed a significantly impaired ratio of familiar-to-novel VEP magnitudes in Grm5$^{+/−}$ mice compared with WT mice ($P = 0.005$) and compared with Grm5$^{+/+}$ mice ($P = 0.001$), but no significant difference between WT and Grm5$^{−/−}$ mice ($P = 0.864$). Baseline day 1 raw VEP magnitude was increased in Grm5$^{−/−}$ mice (WT: $88 ± 7 \mu V$, Grm5$^{−/−}$: $71 ± 4 \mu V$, Grm5$^{+/−}$: $136 ± 25 \mu V, P = 0.013$, one-way ANOVA; post hoc Grm5$^{−/−}$ vs. WT, $P = 0.020$; post hoc Grm5$^{−/−}$ vs. Grm5$^{+/−}$, $P = 0.005$). In summary, SRP was impaired in Grm5$^{−/−}$ mice but not in Grm5$^{+/−}$ mice, measured both by the ability to distinguish familiar from novel stimulus on day 6 and by the growth of VEPs from day 1 to day 6.

The finding of deficient SRP in Grm5 null mice, but not Grm5$^{−/−}$ mice, prompted us to study the effect of CTEP treatment on SRP induction in WT mice. Mice were treated chronically every 48 h with CTEP or vehicle and continued throughout the duration of the 6-d SRP protocol from P30 to P35 (Fig. 4E), the same treatment regime that impaired ocular dominance plasticity. There was no difference in the magnitude of SRP between vehicle-treated and CTEP-treated mice ($P = 0.329$, treatment × day interaction, two-way repeated-measures ANOVA) (Fig. 4F and G), and no difference in the ability to discriminate novel from familiar stimuli on test day 6 ($P = 0.570$, Student t test).
mutant (24), that a partial reduction of signaling μGrm5 mouse, impaired deprived-stores. Grm5 (WT) littermates. All experiments were performed on male Grm5 mice. Although acute inhibition of Grm5 by itself has little effect, modulatory augmentation of phospholipase C signaling has been shown to promote LTD in vitro and synaptic depression in vivo at layer 2/3 synapses in visual cortex (47, 48). Chronic down-regulation of PLC-dependent signaling might have the opposite effect, for example, by altering intracellular Ca2+ stores. Another possibility, not involving the canonical Gq11 signaling pathway, relates to regulation by Grm5 of local synaptic protein synthesis (49) via activation of a Ras-ERK-MAP kinase pathway (50, 51). Chronic inhibition of ERK (52) and mRNA translation (53) also interfere with ocular dominance plasticity. Consistent with this hypothesis, genetic deletion of the mRNA translation repressor fragile X mental retardation protein (FMRP), which boosts basal protein synthesis, is sufficient to restore deprived-eye depression and normal ocular dominance plasticity in the Grm5−−/− mice (24).

Loss of FMRP is the cause of fragile X syndrome, the most common inherited form of human intellectual disability and autism. A core pathophysiological mechanism is excessive protein synthesis downstream of a Grm5-dependent signaling pathway. Inhibitors of this pathway, including CTEP (26), have been shown to correct diverse fragile X phenotypes in multiple animal models (54). In most assays, partial inhibition of Grm5 does not affect WT animals; however, as we have shown here, ocular dominance plasticity is a clear exception. Our data suggest that ongoing signaling at Grm5 during a critical period establishes biochemical conditions that are permissive for activity-dependent sculpting of excitatory synapses via the mechanism of NMDAR-dependent LTD.

Discussion

An interesting feature of early postnatal neocortical development is an increase in group 1 mGluR signaling that, in visual cortex, coincides with increased sensitivity to MD (19). Based on this correlation and on a theory of synaptic plasticity, it was proposed that postsynaptic mGluR signaling might serve as a trigger for homosynaptic depression at glutamatergic synapses (20). Although subsequent research showed that LTD can indeed be triggered by the study of LTD in mutant and treated WT mice (Fig. 2). In layer 4, both approaches to chronically inhibiting mGluR5 produced a clear deficit in NMDAR-dependent LTD, a synaptic modification that uses the same mechanisms of postsynaptic AMPAR modification as deprived-eye depression (4). Interestingly, layer 3 LTD, which has different signaling requirements (18) and is expressed via a presynaptic homosynaptic-dependent mechanism (6), was unaffected by mGluR5 inhibition (Fig. 2), consistent with previous findings (23). Although layer 6 was not examined explicitly in the present study, we expect that LTD in this layer also would be disrupted by treatment, given the evidence that it is induced by activation of mGluR5 rather than NMDARs (17, 18).

Although it would be straightforward to relate deficits in deprived-eye depression and LTD in layer 6, the coordinated deficits in layer 4 synaptic depression present more of a conundrum. In layer 4, LTD is unaffected by acute pharmacological inhibition of mGluR5, mGluR5-dependent signaling pathways, or protein synthesis (Fig. 2) (17, 18). One appealing hypothesis is that chronic inhibition of mGluR5 affects the activity-dependent NMDAR NR2B-to-NR2A subunit switch that occurs postnatally in visual cortex (41–43). Similar to what we observe after chronic inhibition of mGluR5, both NMDAR-dependent LTD and deprived-eye depression are impaired in layer 4 of Grin2a null and heterozygous mice (9). Normal sensory experience during early life drives the change in NMDAR subunit composition, and there is evidence from hippocampus and layer 2/3 of visual cortex that the functional expression of NR2A-containing receptors is triggered by activation of mGluR5 (29); however, our failure to observe a difference in the NMDA EPSC decay kinetics in layer 4 neurons after chronic CTEP suggests that this subunit switch likely occurred normally in treated animals (Fig. 3). We also note that another phenotype caused by reduced NR2A expression, enhanced non-deprived-eye potentiation during 3 d of MD (9), was not observed after chronic inhibition of mGluR5 (Fig. 1). Taken together, these findings argue against the hypothesis that an impaired NR2B-to-NR2A subunit switch is the basis for the impaired deprived-eye depression in layer 4.

Other overt changes in NMDARs appear to be ruled out by the findings of a normal AMPAR/NMDA ratio (Fig. 3) and normal SRP in the Grm5+/− and CTEP-treated WT mice (Fig. 4). We did observe a striking impairment in SRP in the full mGluR5 knockout, however. SRP shares many mechanisms with canonical LTP (36), and NMDAR-dependent LTP is severely impaired in the Grm5+/− hippocampus (44) and layer 4 of sensory neocortex (27), so this finding is not surprising.

mGluR5 also has been implicated in LTD of excitatory synapses onto fast-spiking GABAergic interneurons in visual cortex (45), and both LTD and deprived-eye depression are sensitive to reduced inhibition in visual cortex (33, 46). However, the manipulations of mGluR5 that caused a deficit synaptic depression in vivo and in vitro had no detectable effect on the ratio of excitation to inhibition in layer 4 principal cells in layer 4 (Fig. 3).

We speculate that the cause of altered synaptic depression in layer 4 following chronic mGluR5 inhibition is related to adjustments in intracellular signaling that occur with a slow time course. Although acute inhibition of mGluR5 by itself has little effect, modulatory augmentation of phospholipase C signaling has been shown to promote LTD in vitro and synaptic depression in vivo at layer 2/3 synapses in visual cortex (47, 48). Chronic down-regulation of PLC-dependent signaling might have the opposite effect, for example, by altering intracellular Ca2+ stores. Another possibility, not involving the canonical Gq11 signaling pathway, relates to regulation by mGlu5 of local synaptic protein synthesis (49) via activation of a Ras-ERK-MAP kinase pathway (50, 51). Chronic inhibition of ERK (52) and mRNA translation (53) also interfere with ocular dominance plasticity. Consistent with this hypothesis, genetic deletion of the mRNA translation repressor fragile X mental retardation protein (FMRP), which boosts basal protein synthesis, is sufficient to restore deprived-eye depression and normal ocular dominance plasticity in the Grm5−−/− mice (24).

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Materials and Methods

Animals and Drug Treatment. Male and female Grm5+/− mice (Jackson Laboratory) were bred on a C57BL/6 background, yielding Grm5−−/−, Grm5+/−, and Grm5+/+ (WT) littermates. All experiments were performed on male littermate controls by an experimenter blind to genotype or CTEP treatment. CTEP (Roche) was formulated as a microosmosphere in vehicle (0.9% NaCl, 0.3% Tween-80). Chronic treatment consisted of once per 48 h dosing at 2 mg/kg (s.c.), as described previously (25). Animals were group-housed and kept on a 12-h light/dark cycle. All experiments were approved by Massachusetts Institute of Technology’s Animal Care and Use Committee in conjunction with National Institutes of Health guidelines.

Electrophysiological Recordings and Western Blotting. Extracellular field potential recordings for LTD experiments were obtained using an interface chamber following standard methods (31). P21–P30 mice were used for comparisons of genotype, and this age was restricted to P21-P25 in CTEP experiments. Intracellular recordings were obtained from layer 4 pyramidal neurons in P21–P25 mice using a submersion chamber. AMPA/NMDA ratio (55), E/I balance (56), and NMDA decay (31) experiments were performed essentially as described previously. VEP electrode implantation, electrophysiological recordings, and analysis were performed as described previously (57, 58). In brief, for ocular dominance plasticity experiments, novel oriented visual stimuli were used for recordings both before and after the 3-d MD period. For SRP experiments, a visual stimulus of specific orientation was presented each day except on the final experimental day, where blocks of a novel oriented stimulus were interleaved. Detailed information on slice preparation, extracellular LTD recordings and analysis, intracellular recordings and analysis, in vivo recordings and analysis, and Western blot analysis is provided in SI Materials and Methods.

Statistics. Significant differences between groups were tested using one-way ANOVA, followed by post hoc Student-Newman-Keuls tests. For experiments comparing two conditions (e.g., CTEP vs. vehicle), the Student t test was used. To test whether significant depression occurred within an experimental group, the...
paired Student t test was used on raw (nonnormalized) field potential magnitudes. For MD experiments, two-way repeated-measures ANOVA was used with treatment and time as factors to determine whether there was significant depression of the contralateral VEP with MD. MANOVA was used to test whether CTEP treatment affected contralateral eye depression and ipsilateral eye potentiation. For all LTD and in vivo experiments, n represents the number of animals. Between one and three slice recordings (LTD) or one or two hemispheres (SRP) were averaged together per animal. For intracellular current recordings, n represents the number of cells. In all figures, * indicates P < 0.05 and error bars indicate SEM. Outliers more than two SDs from the mean were excluded.

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