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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.**

mGluR5 | long-term depression | NMDA | visual cortical plasticity

**T**emporary monocular deprivation (MD) sets in motion synaptic changes in visual cortex that result in impaired vision through the deprived eye. The primary cause of visual impairment is depression of excitatory thalamocortical synaptic transmission in layer 4 of visual cortex (1–3). The study of long-term depression (LTD) of synapses, elicited in vitro by electrical or chemical stimulation, has revealed many of the mechanisms involved in deprived-eye depression (4). In slices of visual cortex, LTD in layer 4 is induced by NMDA receptor (NMDAR) activation and expressed by posttranslational modification and internalization of AMPA receptors (AMPA) (5, 6). MD induces identical NMDAR-dependent changes in AMPARs, and synaptic depression induced by deprivation in vivo occludes LTD in visual cortex ex vivo (6–8). Manipulations of NMDARs and AMPAR trafficking that interfere with LTD also prevent the effects of MD (7, 9–11).

Although NMDAR-dependent LTD is widely expressed in the brain (12, 13), it is now understood that different circuits use different mechanisms for long-term homosynaptic depression (14). For example, in the CA1 region of hippocampus, synaptic activation of either NMDARs or metabotropic glutamate receptor 5 (mGluR5) induces LTD. In both cases, depression is expressed postsynaptically as a reduction in AMPARs, but these forms of LTD are not mutually occluding and have distinct signaling requirements (15). A defining feature of mGluR5-dependent postsynaptic LTD in CA1 is a requirement for the immediate translation of synaptic mRNAs (16). In visual cortex, there is evidence that induction of LTD in layers 2–4 requires NMDAR activation, whereas induction of LTD in layer 6 requires activation of mGluR5 (17, 18).

The hypothesis that mGluRs, in addition to NMDARs, play a key role in visual cortical plasticity can be traced back more than 25 y to observations that glutamate-stimulated phosphoinositide turnover, mediated in visual cortex by mGluR5 coupled to phospholipase C, is elevated during the postnatal period of heightened sensitivity to MD (19). Early attempts to test this hypothesis were inconclusive owing to the use of weak and nonselective orthosteric

compounds (20–22); however, subsequent experiments did confirm that NMDAR-dependent LTD occurs normally in layers 2/3 of visual cortex in *Gm5* 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 *Gm5*<sup>+/−</sup> 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 *Gm5*<sup>+/−</sup> 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.

## Results

**Chronic Inhibition of mGluR5 Signaling Impairs Ocular Dominance Plasticity.** Our experiments were motivated by the finding of impaired ocular dominance plasticity in *Gm5*<sup>+/−</sup> mice (Fig. 1 A–C). This finding was surprising on two counts. First, other than ocular

## 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  $\times$  treatment interaction, two-way repeated-measures ANOVA) (Fig. 1E–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*<sup>+/-</sup> mice, indicate that a threshold level of mGluR5 signaling during postnatal development is necessary for ocular dominance plasticity in visual cortex.

### LTD in Layer 4 of Visual Cortex Is Impaired in *Grm5* Mutant Mice.

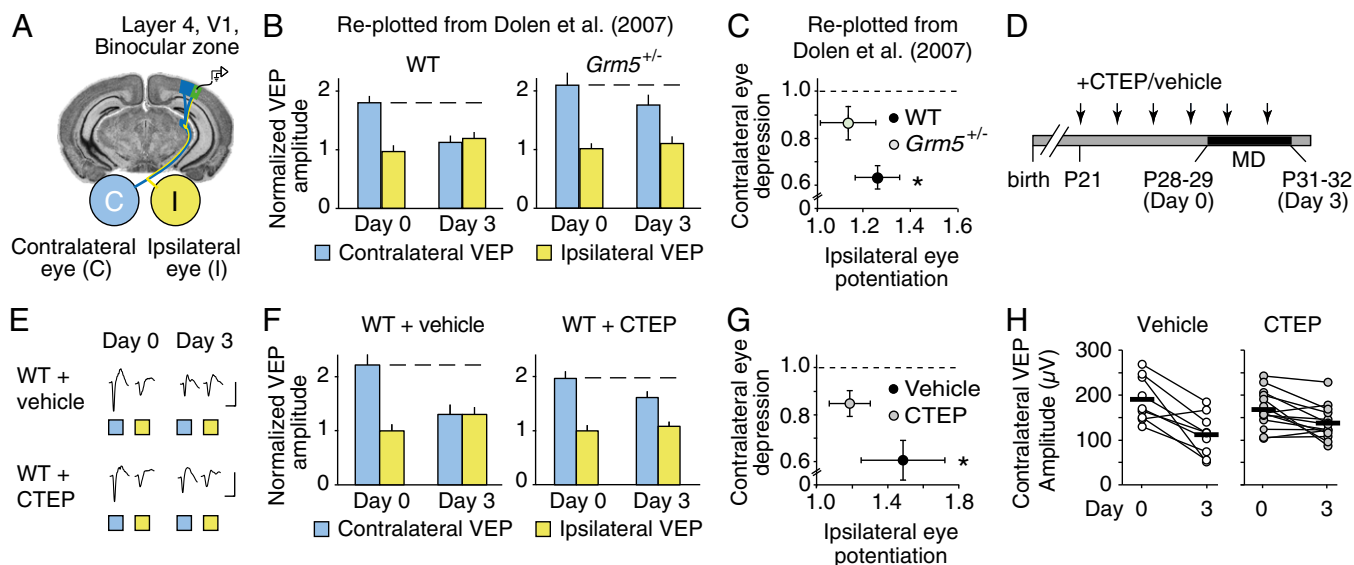
Low-frequency stimulation (LFS; 900 pulses at 1 Hz) induces NMDAR-dependent LTD in visual cortex (5). In layer 4, this LTD is mediated by AMPAR internalization (6), as is deprived eye depression after MD (7, 10, 11). The finding of impaired ocular dominance plasticity in the *Grm5*<sup>+/-</sup> mice led us to ask whether LTD was similarly affected. To address this question, we electrically stimulated white matter of visual cortical slices using a standard LFS LTD induction protocol and recorded extracellular field potentials from layer 4. We observed deficient LTD in *Grm5*<sup>-/-</sup> and *Grm5*<sup>+/-</sup> slices compared with WT littermate controls ( $P = 0.012$ , one-way ANOVA; post hoc tests: WT vs. *Grm5*<sup>-/-</sup>,  $P = 0.012$ ; WT vs. *Grm5*<sup>+/-</sup>,  $P = .033$ ) (Fig. 24). There was no significant difference in LTD magnitude between *Grm5*<sup>-/-</sup> and *Grm5*<sup>+/-</sup> mice ( $P = 0.450$ ).

We also examined LFS LTD in layer 3, and confirmed the findings of a previous study (23) of no deficit in *Grm5*<sup>-/-</sup> or *Grm5*<sup>+/-</sup> slices compared with WT slices ( $P = 0.936$ , one-way ANOVA) (Fig. 2B).

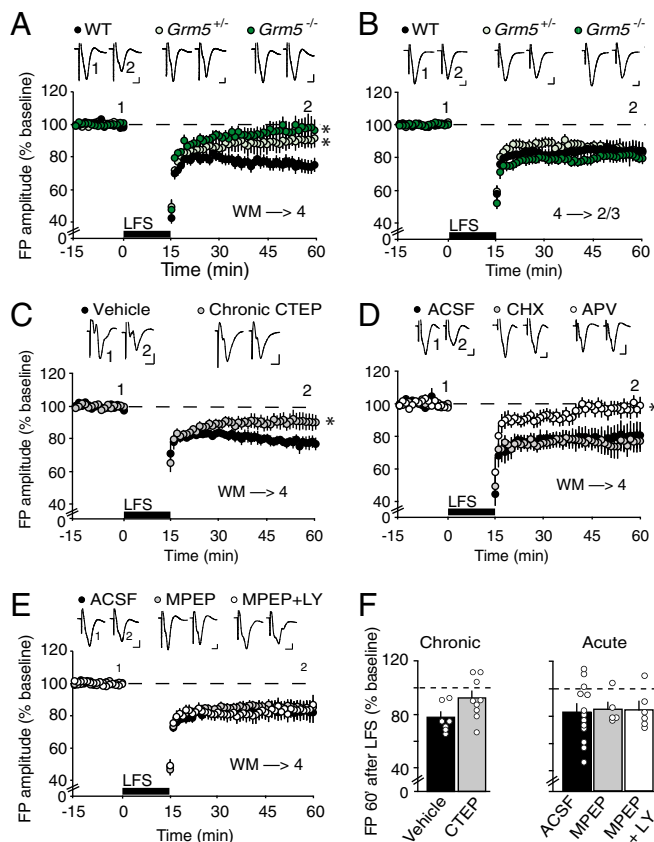
### LTD in Layer 4 Is Disrupted by Chronic, but Not Acute, mGluR5

**Inhibition.** The reduction of layer 4 LTD in the *Grm5* mutant correlates with the impaired deprived-eye depression observed in vivo. To investigate whether this LTD phenotype, like disrupted ocular dominance plasticity, also arises from reduced mGluR5 signaling during postnatal life, we treated mice with CTEP (2 mg/kg s.c.) every other day for 7–11 d from P14 until slice recording at P21–P25. We found that chronic inhibition of mGluR5 significantly reduced the magnitude of LTD in layer 4 of visual cortex in WT mice ( $P = 0.047$ , Student  $t$  test) (Fig. 2C).

Previous work had shown that synaptic depression in layer 4 is mediated by NMDAR-dependent modification of postsynaptic AMPARs. In hippocampus, mGluR5-dependent and NMDAR-dependent forms of LTD are distinct and nonoccluding; thus, we examined the effects of acute pharmacologic manipulations on



**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) In 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<sup>+/-</sup>* mice display deficient deprived-eye depression. Data are replotted from Dölen 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. (Scale bars: 100  $\mu$ V, 100 ms.) (F) MD-induced depression of the contralateral eye-driven VEP is impaired with CTEP compared with vehicle treatment. 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,  $191 \pm 17 \mu$ V; post-MD,  $112 \pm 16 \mu$ V; CTEP contralateral VEP: pre-MD,  $168 \pm 11 \mu$ V; post-MD,  $138 \pm 10 \mu$ V. Not plotted: vehicle ipsilateral VEP: pre-MD,  $86 \pm 11 \mu$ V; post-MD,  $112 \pm 12 \mu$ V; CTEP ipsilateral VEP: pre-MD,  $85 \pm 9 \mu$ V; post-MD,  $92 \pm 7$ . Error bars indicate SEM.



**Fig. 2.** NMDAR-dependent LFS-LTD is impaired in layer 4 with genetic reduction and pharmacologic inhibition of mGluR5. (A) LTD induced by stimulation of white matter and recording in layer 4 is significantly reduced in *Grm5*<sup>-/-</sup> and *Grm5*<sup>+/-</sup> mice. WT: 74.6 ± 3.9% of baseline, *n* = 8 animals (17 slices); *Grm5*<sup>+/-</sup>: 90.8 ± 4.8%, *n* = 6 (9 slices); *Grm5*<sup>-/-</sup>: 97.2 ± 6.6%, *n* = 6 (13 slices). (B) The magnitude of LTD is similar in layer 2/3 across genotypes. WT: 83.9 ± 5.5%, *n* = 6 (11 slices); *Grm5*<sup>+/-</sup>: 83.5 ± 4.3%, *n* = 4 (11 slices); *Grm5*<sup>-/-</sup>: 80.9 ± 5.9%, *n* = 7 (13 slices). (C) Chronic mGluR5 inhibition reduced the magnitude of LFS-induced LTD in layer 4 in WT mice. WT/Vehicle: 77.3 ± 4.1%, *n* = 7 (13 slices); WT/CTEP: 91.8 ± 5.0%, *n* = 9 (13 slices). (D) LFS-LTD in layer 4 is NMDAR-dependent and not protein synthesis-dependent in WT animals. Artificial cerebrospinal fluid (ACSF): 80.2 ± 8.0%, *n* = 4 (9 slices); D-APV: 97.2 ± 6.4%, *n* = 5 (8 slices); cycloheximide: 77.2 ± 6.8%, *n* = 6 (10 slices). (E) Acute inhibition of group 1 mGluRs did not affect LFS-LTD. WT: 82.8 ± 6.0%, *n* = 13 (18 slices); MPEP: 84.8 ± 5.0%, *n* = 5 (11 slices); MPEP + LY367385: 84.4 ± 6.2%, *n* = 6 (13 slices). (F) Summary of LTD experiments. For all figures, displayed traces were averaged across all experiments. Error bars indicate SEM. (Scale bars: 0.2 mV, 50 ms.)

layer 4 LTD. We found that the LTD was indeed blocked by 50  $\mu$ M D-(-)-2-amino-5-phosphonovaleric acid (D-APV), an NMDAR antagonist ( $P = 0.956$ , pre- and post-LFS, paired Student  $t$  test) (Fig. 2D), but not by 60  $\mu$ M cycloheximide, 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  $\mu$ M) had no effect on LTD.

Under some experimental conditions, blockade of mGluR-dependent LTD in the hippocampus requires inhibition of both mGluR5 and mGluR1 (28); thus, we also tested whether simultaneous inhibition of both group 1 mGluRs, using MPEP and (S)-(+)- $\alpha$ -amino-4-carboxy-2-methylbenzeneacetic acid (LY367385; 100  $\mu$ M), 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 impairs 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. 2*F*. 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.

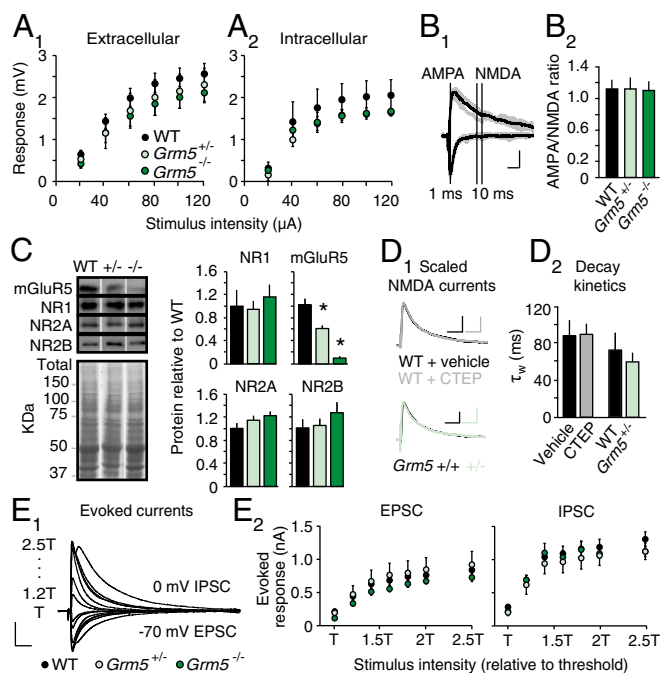
**NMDAR Function and Inhibition Are Unaffected by Chronic Inhibition of mGlu5.** Because genetic knockdown and chronic pharmacologic inhibition of mGluR5 resulted in impaired NMDAR-dependent plasticity in vivo and in vitro, we tested whether NMDARs are functionally impaired in *Grm5* mutants. We first confirmed that basal synaptic transmission, driven mainly by AMPAR-mediated currents, was normal in *Grm5*<sup>+/-</sup> and *Grm5*<sup>-/-</sup> 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-mediated currents were isolated in layer 4 neurons, and showed no difference in *Grm5*<sup>+/-</sup> or *Grm5*<sup>-/-</sup> 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*<sup>+/-</sup>, and *Grm5*<sup>-/-</sup> 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*<sup>-/-</sup> 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 LFS-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*<sup>+/-</sup> or *Grm5*<sup>-/-</sup> 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 ( $R_m$ ) of layer 4 neurons [ $R_m$  (M $\Omega$ ): WT,  $94.6 \pm 11.1$ ; *Grm5*<sup>+/-</sup>,  $91.2 \pm 21.8$ ; *Grm5*<sup>-/-</sup>,  $108.9 \pm 16.9$ ; vehicle,  $108.8 \pm 24.7$ ; CTEP,  $91.1 \pm 13.5$ ].

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





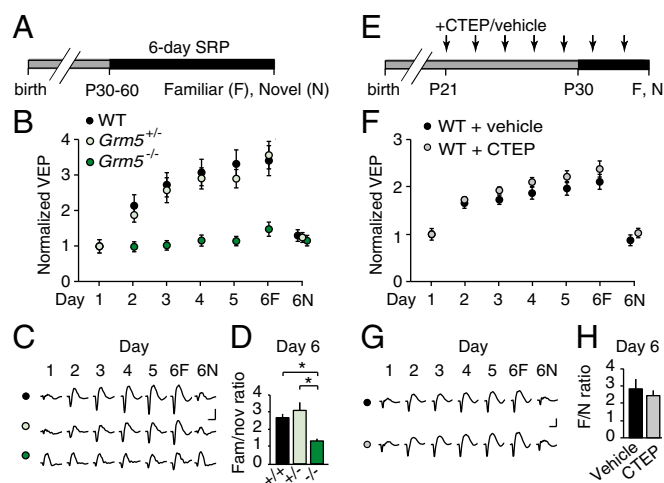
**Fig. 3.** NMDAR function is normal in layer 4 with chronic mGluR5 down-regulation. I/O functions from (A<sub>1</sub>) extracellular field potential LTD experiments ( $n = 6-8$ ) and (A<sub>2</sub>) intracellular voltage clamp recordings (WT,  $n = 7$ ;  $Grm5^{+/-}$ ,  $n = 8$ ;  $Grm5^{-/-}$ ,  $n = 7$ ) showed no change in basal synaptic transmission in  $Grm5^{+/-}$  or  $Grm5^{-/-}$  mice compared with WT mice. (B<sub>1</sub>) The AMPA/NMDA ratio in layer 4 was calculated by comparing AMPA-only responses and NMDA-only responses. The AMPA-only component of the response at +40 mV was taken from a 1-ms window corresponding to the peak at -70 mV, and the NMDA-only response was taken from a 10-ms window at +40 mV, where no AMPA response was present. (Scale bars: 25 ms, 50 pA.) (B<sub>2</sub>) The AMPA/NMDA ratio was normal in  $Grm5^{+/-}$  and  $Grm5^{-/-}$  neurons (WT:  $1.12 \pm 0.10$ ,  $n = 8$ ;  $Grm5^{+/-}$ :  $1.12 \pm 0.11$ ,  $n = 10$ ;  $Grm5^{-/-}$ :  $1.10 \pm 0.14$ ,  $n = 11$ ). (C) Levels of NR1 protein were normal in  $Grm5^{+/-}$  and  $Grm5^{-/-}$  visual cortex slices (WT:  $100.0 \pm 23.8\%$  of WT;  $Grm5^{+/-}$ :  $93.8 \pm 14.2\%$ ;  $Grm5^{-/-}$ :  $115.1 \pm 20.5\%$ ,  $n = 11$ ). Levels of mGluR5 protein were reduced (WT:  $100.0 \pm 10.4\%$ ;  $Grm5^{+/-}$ :  $59.9 \pm 5.2\%$ ;  $Grm5^{-/-}$ :  $9.8 \pm 3.2\%$ ,  $n = 6$ ). Levels of NR2A protein and NR2B protein were normal (NR2A WT:  $100.0 \pm 9.3\%$ ;  $Grm5^{+/-}$ :  $114.1 \pm 7.7\%$ ;  $Grm5^{-/-}$ :  $121.9 \pm 7.1\%$ ; NR2B WT:  $100.0 \pm 14.9\%$ ;  $Grm5^{+/-}$ :  $104.2 \pm 11.6\%$ ;  $Grm5^{-/-}$ :  $125.9 \pm 18.0\%$ ;  $n = 12$ ). (D<sub>1</sub>) NMDA currents were isolated at +40 mV in the presence of NBQX. (Scale bars: 50 ms, 50 pA.) (D<sub>2</sub>) The weighted decay constant of NMDA currents was similar in WT mice treated chronically with vehicle and those treated with CTEP (vehicle  $\tau_w = 88.3 \pm 16.8$ ,  $n = 9$ ; CTEP  $\tau_w = 89.8 \pm 11.5$ ,  $n = 10$ ), and between WT mice and  $Grm5$  mutants (WT  $\tau_w = 72.5 \pm 18.8$ ,  $n = 4$ ;  $Grm5^{+/-}$   $\tau_w = 59.8 \pm 10.6$ ,  $n = 6$ ). (E<sub>1</sub>) Evoked IPSCs and EPSCs were isolated in layer 4 neurons by holding cells at 0 mV and -70 mV, respectively. White matter stimulation yielded a threshold stimulation intensity required to evoke responses in layer 4 (T). The amplitude of evoked IPSCs and EPSCs were recorded as a function of stimulation intensity relative to T. (Scale bars: 200 ms, 50 pA.) There was no effect of  $Grm5$  genotype on (E<sub>2</sub>) evoked EPSC amplitude or evoked IPSC amplitude ( $n = 9-13$ ). Error bars indicate SEM.

inhibition was functionally altered in visual cortex by mGluR5 knock-down. We measured evoked EPSCs 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  $\times$  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:  $88 \pm 7 \mu V$ ,  $Grm5^{+/-}$ :  $71 \pm 4 \mu V$ ;  $Grm5^{-/-}$ :  $136 \pm 25 \mu V$ ,  $P = 0.013$ , 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, beginning at P21 and continuing throughout the duration of the 6-d SRP protocol from P30 to P35 (Fig. 4E), the same treatment regimen 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  $\times$  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)



**Fig. 4.** SRP persists in  $Grm5^{+/-}$  and CTEP-treated mice, but is impaired in  $Grm5^{-/-}$  mice. (A) SRP was induced by presentation of a familiar stimulus (45°; F) on 6 consecutive days, followed by interleaved presentation of a novel stimulus (135°; N) on test day 6. (B) SRP (normalized to day 1 VEP within group) was significantly impaired in  $Grm5^{-/-}$  mice (WT,  $n = 14$ ;  $Grm5^{+/-}$ ,  $n = 9$ ;  $Grm5^{-/-}$ ,  $n = 10$ ). (C) Averaged VEPs across test days. (D)  $Grm5^{-/-}$  mice showed significant impairments in distinguishing familiar stimuli from novel stimuli (F/N ratios on test day 6: WT,  $2.7 \pm 0.2$ ;  $Grm5^{+/-}$ ,  $3.1 \pm 0.5$ ;  $Grm5^{-/-}$ ,  $1.3 \pm 0.1$ ). (E) SRP was induced beginning on P30 after chronic CTEP or vehicle treatment, which began at P21. (F) SRP magnitude was not significantly different between CTEP-treated and vehicle-treated WT mice. (G) Averaged VEPs across test days. (H) Chronic CTEP did not affect the ability to distinguish familiar from novel stimuli (vehicle:  $2.9 \pm 0.6$ ,  $n = 8$ ; CTEP:  $2.5 \pm 0.3$ ,  $n = 7$ ). (Scale bars: 100 ms, 100  $\mu V$ .) Error bars indicate SEM.

(Fig. 4H). Baseline day 1 raw VEP magnitude was not affected by CTEP treatment (vehicle:  $159 \pm 10$   $\mu$ V, CTEP:  $142 \pm 17$   $\mu$ V;  $P = 0.402$ , Student  $t$  test). Taken together, these data indicate that partial inhibition of mGluR5 signaling during development selectively impairs the mechanism of NMDAR-dependent synaptic weakening.

## 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 a consequence of group 1 mGluR activation (38, 39), surprisingly little progress has been made in establishing a role for postsynaptic mGluRs in visual cortical plasticity in vivo.

Here we confirm pharmacologically what was previously shown in the *Gm5* mutant (24), that a partial reduction of signaling via mGluR5 interferes with the effects of MD in layer 4 of visual cortex (Fig. 1). CTEP treatment during the period of heightened sensitivity to MD, beginning at  $\sim$ P21 (40), was sufficient to reproduce the phenotype observed in the *Gm5*<sup>+/-</sup> mouse, impaired deprived-eye depression. The likely basis for this deficit in vivo was revealed 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 endocannabinoid-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 pharmacologic 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/NMDAR ratio (Fig. 3) and normal SRP in the *Gm5*<sup>+/-</sup> 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 *Gm5*<sup>-/-</sup> hippocampus (44) and layer 4 of sensory neocortex (27), so this finding is not surprising.

mGluR5 also has been implicated in LTP 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  $\text{Ca}^{2+}$  stores. Another possibility, not involving the canonical Gq11 signaling pathway, relates to regulation by mGluR5 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 *Gm5*<sup>+/-</sup> 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 an mGluR5-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 mGluR5 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 mGluR5 during a critical period establishes biochemical conditions that are permissive for activity-dependent sculpting of excitatory synapses via the mechanism of NMDAR-dependent LTD.

## Materials and Methods

**Animals and Drug Treatment.** Male and female *Gm5*<sup>+/-</sup> mice (Jackson Laboratory) were bred on a C57BL/6 background, yielding *Gm5*<sup>-/-</sup>, *Gm5*<sup>+/-</sup>, and *Gm5*<sup>+/+</sup> (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 microsuspension 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 procedures 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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