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The regulatory role of long-term depression in juvenile and adult mouse ocular dominance plasticity

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The study of experience-dependent ocular dominance (OD) plasticity has greatly contributed to the understanding of visual development. During the critical period, preventing input from one eye results in a significant impairment of vision, and loss of cortical responsivity via the deprived eye. Residual ocular dominance plasticity has recently been observed in adulthood. Accumulating evidence suggests that OD plasticity involves *N*-methyl-$_D$-aspartate receptor (NMDAR)-dependent long-term depression (LTD). Here we report that the administration of a selective LTD antagonist prevented the ocular dominance shift during the critical period. The NMDAR co-agonist D-serine facilitated adult visual cortical LTD and the OD shift in short-term monocularly deprived (MD) adult mice. When combined with reverse suture, D-serine proved effective in restoring a contralaterally-dominated visual input pattern in long-term MD mice. This work suggests LTD as a key mechanism in both juvenile and adult ocular dominance plasticity, and D-serine as a potential therapeutic in human amblyopic subjects.

The modification of cortical ocular dominance (OD) represents a well-characterized example of experience-dependent modification of brain circuitry. During a well-defined "critical period" during early postnatal life, visual cortical circuitry is extremely susceptible to various forms of deprivation. Monocular deprivation (MD) of visual input during the critical period results in life-long impairment in the visual acuity of the deprived eye (amblyopia). Some weak forms of ocular dominance plasticity have been observed to last through adulthood, but the effects are less pronounced, and/or require longer periods of deprivation[1–3]. The mechanisms underlying the age-related changes in developmental plasticity continue to be the subject of intensive study. Abundant evidence points to the importance of an appropriate level of cortical inhibition[4], prior visual exposure[5], and a role for axonal sprouting[6].

N-methyl-$_D$-aspartic acid receptor (NMDAR) regulated neurotransmission has been suggested to play an important role in OD plasticity in both juvenile and adult rodents. Pharmacological blockade[7,8] or genetic deletions[9] of NMDAR prevent the OD shift after MD during the critical period. Competitive NMDAR antagonists block adult ocular dominance plasticity as well[10]. Two forms of NMDAR-dependent neurotransmission have been widely associated with OD plasticity, namely long-term depression (LTD) and long-term potentiation (LTP).

Recent studies have favored LTD over LTP as a key mechanism for juvenile OD plasticity[11–14]. To probe the functional significance of LTD in juvenile and adult ocular dominance plasticity, we used a specific LTD blocking peptide GluR2$_{3Y}$. GluR2$_{3Y}$, derived from GluR2 carboxyl tail (869YKEGYNVYG877), blocks the expression of LTD in many brain areas, and has been used to study the role of LTD in several learning related behaviours[15–19].

We also examined the functional importance of LTD in adult ocular dominance plasticity by application of the NMDAR co-agonist D-serine. D-serine has been implicated an important regulatory role in NMDA transmission, synaptic plasticity and development[20]. Previous studies have indicated that the NMDAR co-agonist site is not saturated under resting conditions, and thus co-agonist binding could potentially modulate NMDAR activity[21,22]. Indeed, Duffy and colleagues showed that exogenous D-serine application could enhance hippocampal LTD and spatial reversal learning in adult mice[23]. Here we examined the effect of D-serine on LTD in adult visual cortical slices *in vitro* and ocular dominance plasticity of adult mice *in vivo*.

Results

Temporal Correlation between LTD and the critical period; blockade of LTD in juvenile cortical slices. To investigate the functional significance of LTD in ocular dominance plasticity, we first examined the strength of

LTD in mice visual cortical slices at different developmental ages. We found that the magnitude of LTD declined gradually throughout early development, and it was not evoked in adulthood. LTD was induced by delivering low-frequency stimulation (LFS, 1 Hz, 900 stimuli) to layer IV, and the magnitude of field excitatory post-synaptic potentials (fEPSPs) evoked in layer II/III were recorded. Age groups studied included pre-critical period (P11–P12; P16–P17), pericritical period (P22–P23; P27–P28), and post-critical period (P41–P43; P83–P95). The magnitudes of depression for each age group tested were as follows: P11–P12: 22.10% (n=6), P16–P17: 17.30% (n=7), P22–P23: 17.20% (n=6), P27–P28: 10.50% (n=5), P41–P43: 8.20% (n=6), P83–P95: 0 (n=5), showing a gradual decrease during early postnatal period (**Fig 1A**).

In order to establish $GluR2_{3Y}$ peptide as a valid visual cortical LTD-blocker, we first investigated its effectiveness in blocking AMPAR endocytosis in visual cortical slices (**Fig 1B, C**). $GluR2_{3Y}$ was rendered membrane permeable by fusing it to the cell membrane transduction domain of the HIV-1 Tat protein (YGRKKRRQRRR) to generate Tat-$GluR2_{3Y}$ as previously described[16]. Visual cortex slices (around P25) were incubated with 1 μM Tat-$GluR2_{3Y}$ for 30 minutes. Chemical LTD was then induced using 20 μM NMDA for 5 minutes. After biotinylation, western blotting was used to measure the density of surface AMPARs. NMDA treatment caused a marked reduction of surface GluR2 subunits both in the absence of Tat-$GluR2_{3Y}$ (81.96 ± 4.42 %, n=3; $p<0.05$) and with pretreatment with scrambled Tat-$GluR2_{3Y}$ (79.06 ± 4.13 %, n=3; $p<0.05$), but did not alter the level of GluR2 subunits in cortical slices pretreated with Tat-$GluR2_{3Y}$ (94.39 ± 6.92 %, n=3; p=0.45). In contrast, NMDA treatment did not change the expression of $GABA_A$ β2/3 on the cell surface (96.19 ± 4.81 %, n=3; p=0.46), nor was this altered by the pretreatment with either Tat-$GluR2_{3Y}$ (108.10 ± 13.00 %, n=3; p=0.56) or scrambled Tat-$GluR2_{3Y}$ (95.91 ± 5.26 %, n=3; p=0.47), suggesting that the peptide is specific for GluR2 subunit of AMPAR (**Fig 1C**). GSK3b, an intracellular protein, was also probed as a control for specific biotinylation on the plasma membrane, but not of intracellular proteins. An example is shown in **Fig 1B**.

Next, we tested the effectiveness of $GluR2_{3Y}$ by performing local field potential recordings in juvenile mouse visual cortical slices. At around P25, LFS reliably induced LTD of extracellularly recorded excitatory postsynaptic potentials (EPSPs) in mouse visual cortex slices (the magnitude of the fEPSPs at 30 min was reduced to 80 ± 3 % of the baseline, n = 5; p = 0.008 compared with the baseline recorded 5 min prior to the start of LFS, **Fig 1D**). This LTD was *N*-methyl-$_D$-aspartate (NMDA) receptor dependant because it was blocked by the NMDA receptor antagonist D-APV at a concentration of 50 μM (n=5; **Fig 1D, F, G**). Bath application of the Tat-$GluR2_{3Y}$ peptide (0.4 μM) for 20 min prior to LFS abolished LTD (96 ± 2 % of baseline, n = 7; p = 0.012 when compared to control LTD; **Fig 1D, F, G**). In contrast, a control peptide, Tat-$GluR2_{3A}$ (Tat-AKEGANVAG, in which the three critical tyrosine residues were replaced with alanines[19]) at the same concentration did not significantly alter the magnitude of LTD (87 ± 6 % of baseline, n = 4; p = 0.23; **Fig 1D, F, G**).

To further confirm that the Tat-$GluR2_{3Y}$ peptide interfered with the regulated endocytosis of postsynaptic AMPARs, we applied the membrane impermeable form of the peptides ($GluR2_{3Y}$ or $GluR2_{3A}$; 100 μg/ml) to postsynaptic neurons under whole-cell recording conditions by including them in the patch electrodes. Consistently, $GluR2_{3Y}$, but not $GluR2_{3A}$, significantly reduced the LFS-induced LTD of excitatory postsynaptic currents (EPSCs) (**Fig 1D, F, G**). We observed no significant change in basal synaptic transmission with either peptide under either field or whole cell recording conditions. In summary, our results indicate that the Tat-$GluR2_{3Y}$ peptide is effective in blocking NMDAR-dependent AMPAR endocytosis and LTD in visual cortical slices.

Prevention of OD shift following MD with systemic administration of the $GluR2_{3Y}$ peptide. We next applied the peptides *in vivo* to examine their effect on the OD shift following MD during the critical period. We administered Tat-$GluR2_{3Y}$ or the control peptide, Tat-$GluR2_{3A}$, via intraperitoneal injection daily during a four day period of MD in mice at the peak of the critical period (P25–P29). Single unit recording was performed to examine visual cortical OD[24]. The control non-deprived group (ND) displayed a contralateral biased distribution of OD scores with a contralateral bias index (CBI) of 0.77 ± 0.01 (n = 5; **Fig 2A**). A four day period of MD shifted the OD toward the open ipsilateral eye (CBI = 0.49 ± 0.05, n = 5; p = 0.007; **Fig 2B**). Thus, these animals displayed the characteristic MD-induced OD shift. Tat-$GluR2_{3Y}$ (10 nmol/g) administration for four days had no influence on OD in non-deprived mice (CBI = 0.79 ± 0.02, n = 5; p = 0.35 compared to that in the control ND mice; **Fig 2C**), but prevented the MD-induced OD shift in MD mice (CBI = 0.74 ± 0.02, n = 5; p = 0.006 compared to MD mice and p = 0.26 compared to ND mice; **Fig 2D, E**). In contrast, the control peptide (Tat-$GluR2_{3A}$, 10 nmol/g), did not affect the normal MD-induced OD shift (**Fig 2F**). $GluR2_{3Y}$ treatment did not influence the spontaneous activity, the stimulus driven visual responsiveness (**Fig. 2G**), or the general receptive field properties of cortical cells as assessed quantitatively (data not shown). Thus, following systemic application, Tat-$GluR2_{3Y}$, but not Tat-$GluR2_{3A}$, specifically prevented the MD-induced OD shift.

Prevention of OD shift following MD with local infusion of GluR2 peptide. To examine more closely the importance of visual cortical LTD in the MD-induced OD shift, we applied the peptides directly to the primary visual cortex by local infusion with osmotic minipumps (**Fig. 3**). Fluorescent imaging demonstrated that FITC-conjugated Tat-$GluR2_{3Y}$ was efficiently delivered to and localized within the primary visual cortex 24 h after implantation of minipumps filled with the peptide at a concentration of 2 μM (**Fig 3A**). We began a four-day period of MD 24 h after implantation of the pumps containing Tat-$GluR2_{3Y}$ or Tat-$GluR2_{3A}$ (2 μM) (**Fig 3B**). After this period, single unit recording was performed to examine visual cortical OD. Consistent with the diffusion of the peptide into the binocular zone shown in **Fig 3A**, robust changes in ocular dominance distribution were detected by electrophysiological recordings (**Fig 3C,D**). The mice treated with Tat-$GluR2_{3Y}$ did not show an OD shift towards the open eye (CBI = 0.76 ± 0.01, n = 5; p = 0.82 compared to ND mice and p = 0.004 compared to MD mice; **Fig 3C**). In contrast, the mice treated with the control Tat-$GluR2_{3A}$ showed a significant OD shift to the open eye (CBI = 0.46 ± 0.01, n = 5; p = 0.005 compared to ND mice, and p = 0.33 compared to MD mice; **Fig 3D**). Taken together, our results using single unit recordings (summarized in **Fig 2H, 3E**) strongly suggest that LTD is required for the MD-induced OD shift, and that *in vivo* blockade of regulated AMPAR endocytosis and/or LTD during the critical period can prevent the ocular dominance shift.

D-serine selectively enhanced LTD in adult mouse cortical slices. Given the evidence demonstrating the importance of LTD in normal visual development[11–14], we went on to ask whether facilitating LTD could enhance ocular dominance plasticity in adult animals. D-serine was used to this end. Low-frequency stimulation (LFS, 1 Hz, 900 stimuli) delivered to layer IV could not induce LTD in layers II/III in adult visual cortical slices (P90–P100, 100±0.1% of baseline, n=3), but the induction of LTD was facilitated by co-application of D-serine (**Fig 4**). Cortical slices were incubated in D-serine (20 μM) for at least 20 minutes of baseline recording before LFS was delivered. D-serine incubation did not affect the baseline activity, but it facilitated the decrease of the fEPSP in the presence of low frequency stimulation (66±12% of baseline, n=4; $p< 0.01$). The effect was stable for more than 30 minutes (**Fig 4A**).

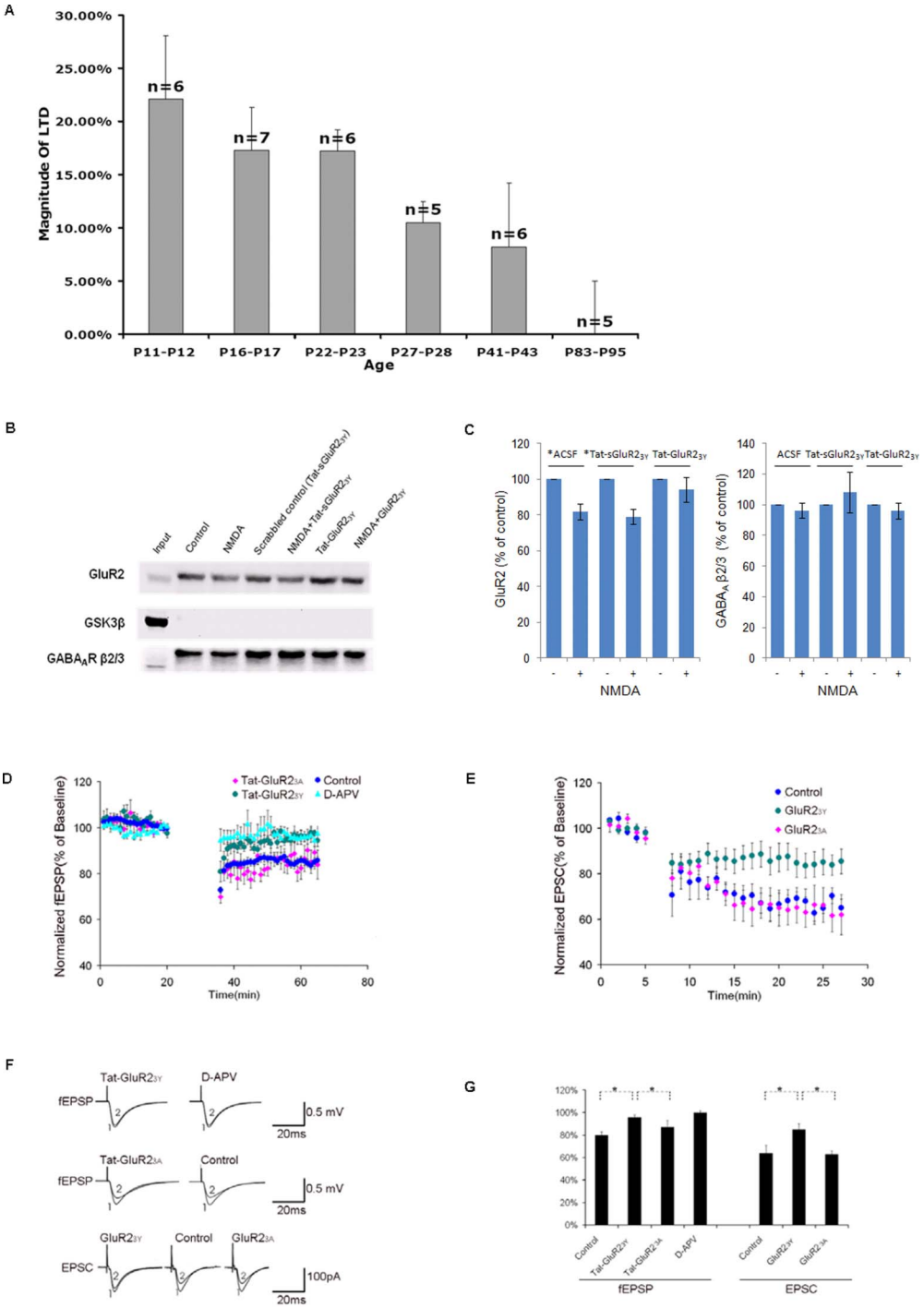

Figure 1 | Correlation between LTD magnitude and the critical period; LTD blockade. (A) Local field potential recording showed that the magnitude of LFS-induced LTD declined with development, and was much diminished in adulthood. (B) Pretreatment of the slices with Tat-GluR2$_{3Y}$ (1 μM; 30 min), but not scrambled control (Tat-sGluR2$_{3Y}$, 1 μM; 30 min), prevented the NMDA-mediated reduction in AMPARs in response to bath application of NMDA (20 μM; 5 min) in visual cortical slices (around P25), without altering the basal level of surface AMPARs, or GABA$_A$ receptors. GSK3b was probed as a control for specific biotinylation of surface proteins. (C) Quantification of surface AMPARs and GABA$_A$ receptors after chemical LTD induction. A marked reduction of surface GluR2 subunits was observed both in the absence of Tat-GluR2$_{3Y}$ (81.96 ± 4.42 % of control, n=3; $p<0.05$) and with pretreatment with scrambled Tat-GluR2$_{3Y}$ (79.06 ± 4.13 % of control, n=3; $p<0.05$), but not with pretreatment with Tat-GluR2$_{3Y}$ (94.39 ± 6.92 % of control, n=3; p=0.45). The expression of GABA$_A$ β2/3 on the cell surface was not changed by NMDA treatment (96.19 ± 4.81 %, n=3; p=0.46), neither Tat-GluR2$_{3Y}$ (108.10 ± 13.00 %, n=3; p=0.56) nor scrambled Tat-GluR2$_{3Y}$ (95.91 ± 5.26 %, n=3; p=0.47) pretreatment altered that effect. Asterisk, $p < 0.05$. Error bars reflect SEM. (D) Bath application of Tat-GluR2$_{3Y}$ blocked NMDAR-dependant LTD of field recordings in primary visual cortical slices (around P25). Red diamond, GluR2$_{3A}$ (n=6); green circle, GluR2$_{3Y}$ (n=7); blue circle, control LTD (n=7); light blue triangle, D-APV treatment (n=5). (D) Inclusion of GluR2$_{3Y}$ (100 μM; n = 7), but not GluR2$_{3A}$ (100 μM; n = 7) in patch electrodes blocked LTD of excitatory postsynaptic currents (EPSCs) in whole-cell recordings. (F) Representative fEPSPs and EPSCs taken just before the LFS (1) and near the end of recording (2) as indicated in both **Fig 1D and 1E**. (G) Summary of results obtained under field recording and whole-cell recording conditions. Each bar represents the average of normalized fEPSPs or EPSCs recorded in the last 5 min under conditions as labeled on the X-axis. Asterisk, $p < 0.05$. Error bar represents SEM.

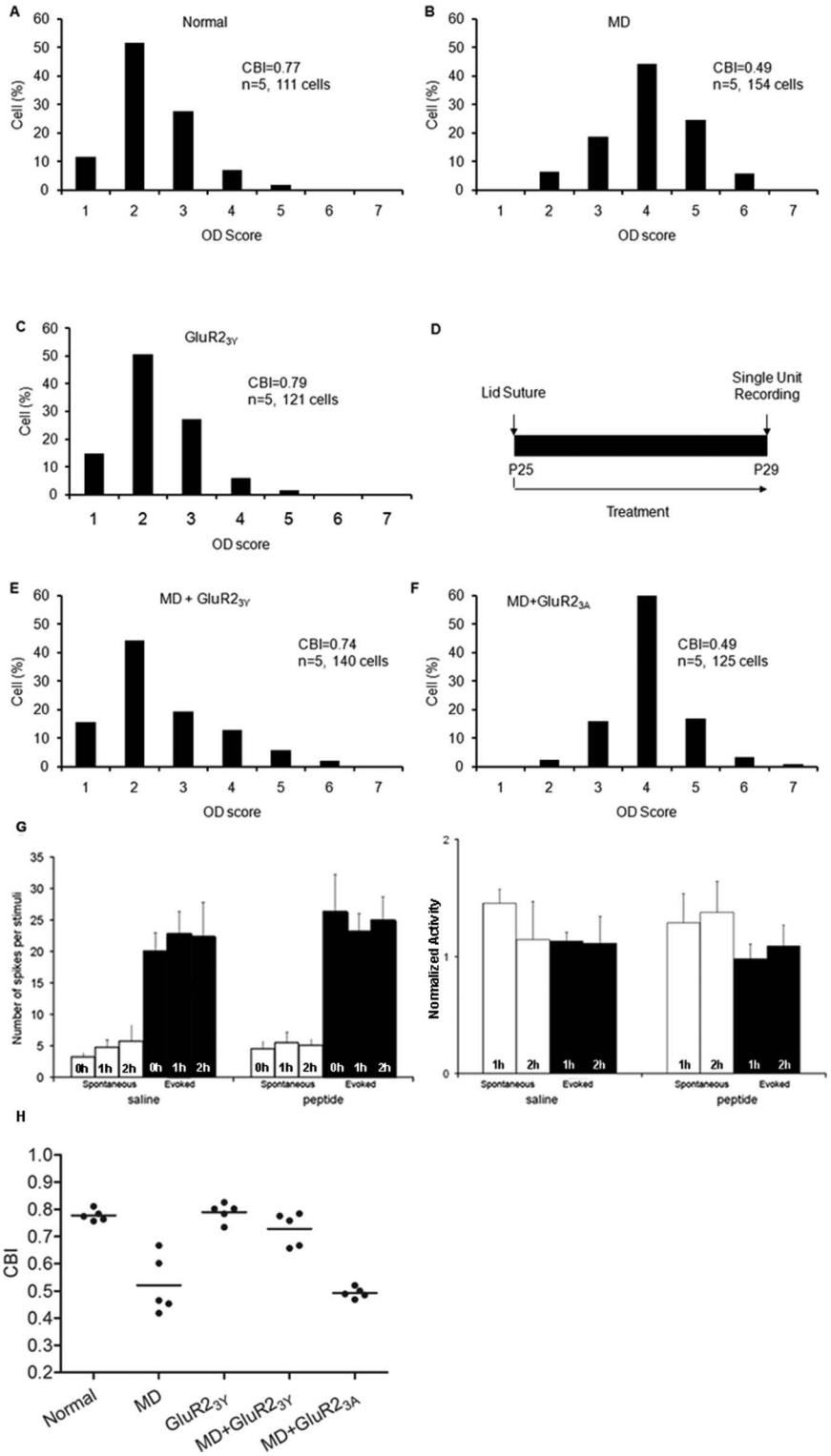

Figure 2 | GluR2$_{3Y}$ peptide administration prevents the ocular dominance shift *in vivo*. (A) The OD distribution favored the contralateral eye in normal non-deprived (ND) mice. (B) Monocular deprivation shifted the distribution towards the ipsilateral eye. (C) Tat-GluR2$_{3Y}$ peptide had no influence on OD in ND mice. (D) Tat-GluR2$_{3Y}$ or Tat-GluR2$_{3A}$ (10 nmol/g; i.p.) was administered daily during the four-day MD period. (E) Tat-GluR2$_{3Y}$ (10 nmol/g; i.p.) prevented the OD shift following MD ($p = 0.26$ compared to nondeprived mice). (F) Tat-GluR2$_{3A}$ did not prevent the OD shift following MD. (G) Tat-GluR2$_{3Y}$ had no acute influence on the spontaneous activity and evoked response in the binocular zone of the primary visual cortex. The number of spikes was counted with or without the presence of the visual stimulus (a moving grating), 1 h or 2 h after i.p. injection of saline or peptide. The Y-axis shows the number of spikes obtained. White bars represent spontaneous activity and blue bars represent evoked activity. Bars from left to right represent before peptide/saline administration; 1 h after administration and 2 h after administration. For statistical analysis, spontaneous and evoked spikes at 1 h and 2 h are normalized against the spontaneous or evoked activity at 0 h at the same site on the same animal. The same normalization was done for animals treated with peptide or saline. (H) Data summary of single-unit recordings in juvenile mice. The dots represent individual animals. The horizontal bar represents the mean of the CBI for each group.

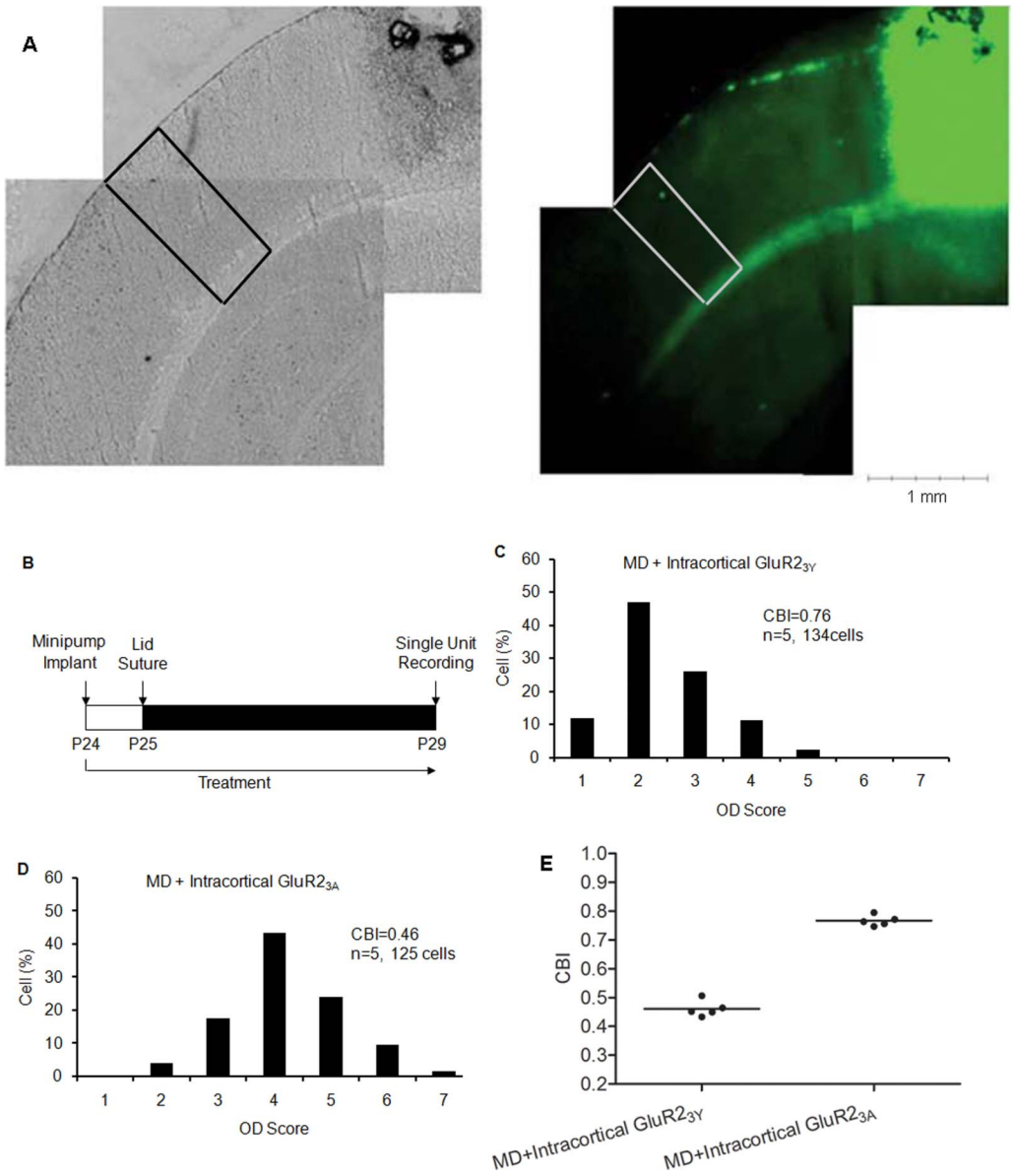

Figure 3 | Local infusion of Tat-GluR2$_{3Y}$ in the primary visual cortex blocks the OD shift. (A) FITC-conjugated Tat-GluR2$_{3Y}$ (2 μM) was delivered using osmotic minipumps. After 24 h, the fluorescent dye was detected throughout the visual cortex, but not in the brain stem or on the contralateral side of the brain. The left panel was taken under transmitted light, the right panel was taken under fluorescence. Scale bar (1 mm) is illustrated at the bottom right. The binocular zone is highlighted. (B) Experimental schedule for intracortical infusion of the peptides. (C) Local infusion of Tat-GluR2$_{3Y}$ prevented the OD shift (0.76 ± 0.01, n = 5; p = 0.82 compared to ND mice and p = 0.004 compared to MD mice). (D) Local infusion of Tat-GluR2$_{3A}$ did not prevent the OD shift (CBI = 0.46 ± 0.01, n = 5; p = 0.005 compared to ND mice, and p = 0.33 compared to MD mice). (E) data Summary of the binocularity index results under the various conditions studied.

However, D-serine did not facilitate the induction of LTP in adult visual cortical slices. (Theta-burst stimulation) TBS did not elicit LTP in adult cortical slices (99±4% of baseline, n=5), nor did any of the concentrations of D-serine from 20 μM up to 200 μM facilitate the induction of LTP (96±6% of baseline, n=5; **Fig 4B**). Thus our results show that D-serine specifically enhances the induction of LTD in adult cortical slices without affecting LTP, making it a useful reagent to study the importance of LTD in adult ocular dominance plasticity.

D-serine enhances ocular dominance plasticity in adult mice. We next examined the effect of D-serine *in vivo* by examining the ocular dominance shift in adult monocularly deprived mice. As others have reported, four days of MD (P90–P94) did not induce a significant ocular dominance shift in adulthood as revealed by single-unit recordings (CBI=0.78±0.01, n=5; p=064, compared to normal adult mice; **Fig 5A, B**). The half life of D-serine in the rodent brain has been reported to be around 12 hours[25]. We adopted an effective dose of 600 mg/kg, as reported by Lipina et al.[26], and administered D-serine at the half-life interval for the purpose of prolonging the duration of the effective therapeutic dose. Consistent with data showing that D-serine does not affect basal activity in brain slices, D-serine administration *in vivo* (600 mg/kg, bid, s.c) alone did not affect the ocular dominance distribution and general visual response properties of cortical neurons (CBI=0.74±0.01, n=5; p=0.03 compared to normal adult mice; **Fig 5C**). However, when challenged with monocular deprivation (**Fig 5D**), D-serine treated mice demonstrated a significant ocular dominance shift (CBI=0.56±0.05, n=5; p<0.01, compared to normal mice; **Fig 5E**). Control saline injections failed to induce the same effect (CBI=0.77±0.01, n=5, p=0.23, compared to normal mice, **Fig 5F**). Quantitative statistical

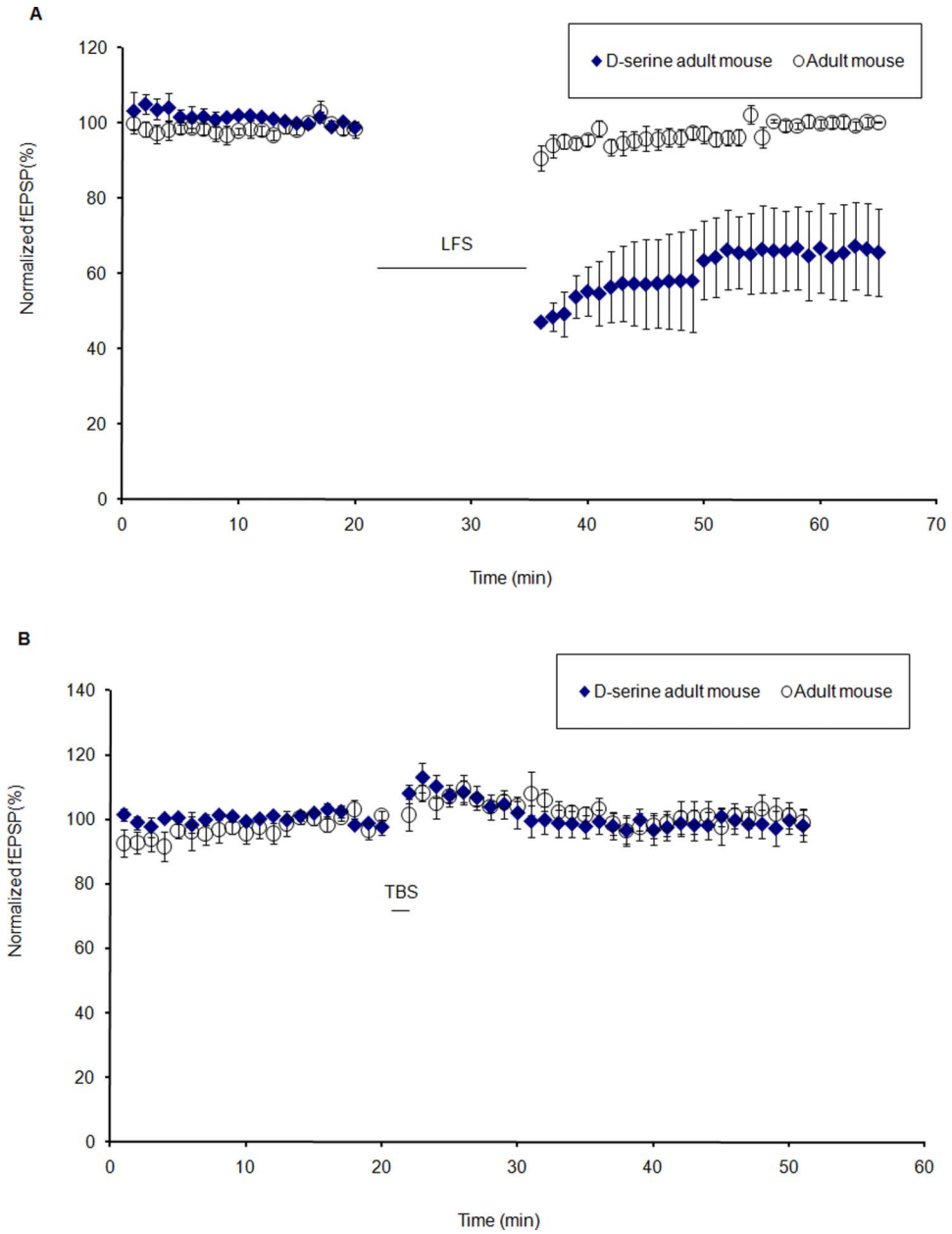

Figure 4 | D-serine selectively facilitates the induction of LTD in adult visual cortical slices. (A) D-serine facilitated LTD in adult visual cortical slices. 20 minutes of D-serine (20 μM) incubation facilitated the induction of LTD (66±12% of baseline, n=4; $p < 0.01$) after delivering low-frequency stimulation (1 Hz, 900 stimuli). Error bars reflect SEM. (B) D-serine did not facilitate LTP in adult visual cortical slices. 20 minutes of D-serine incubation (concentrations from 20μM up to 200 μM) did not affect the induction of LTP (98±4%, n=5) after delivering theta-burst stimulation.

comparison of CBI among adult mice of different treatment groups showed that D-serine enhanced adult ocular dominance plasticity (**Fig 5H**).

To more closely evaluate whether D-serine enhances adult ocular dominance plasticity through the facilitation of LTD, we applied $GluR2_{3Y}$ in D-serine treated monocularly deprived adult mice. Mice that received both $GluR2_{3Y}$ (10 nmol/g, i.p.) and D-serine (600 mg/kg, bid, s.c.) treatment demonstrated a contralateral-biased ocular dominance distribution (CBI=0.68, n=4, **Fig 5G**), indicating that $GluR2_{3Y}$ prevented D-serine-induced OD shift.

Intracortical Infusion of D-serine enhances visual cortical plasticity in adult mice. To rule out any potential influence from other components of the visual pathway, we examined the effect of D-serine applied directly to the primary visual cortex by local infusion through osmotic minipumps (**Fig. 6**). The pumps, containing either D-serine (50μM) or saline, were implanted one day prior to lid suture (P89). After four days of monocular deprivation (P90–P94), single unit recordings of the D-serine treated group showed a significant OD shift towards the open eye (CBI=0.47±0.01, n=3; $p<0.01$; **Fig. 6B**), while the saline treatment failed to induce similar effects (CBI=0.78±0.01, n=3; **Fig. 6C**). Thus, intracortical infusion and systemic administration of the drug induced similar OD shifts (**Fig. 5E, 6B**), indicating that D-serine enhances ocular dominance plasticity via a mechanism operating in the visual cortex.

D-serine administration in adulthood, together with reverse suture, restores contralaterally-biased visual input in long-term monocularly deprived mice. Since D-serine facilitated adult visual

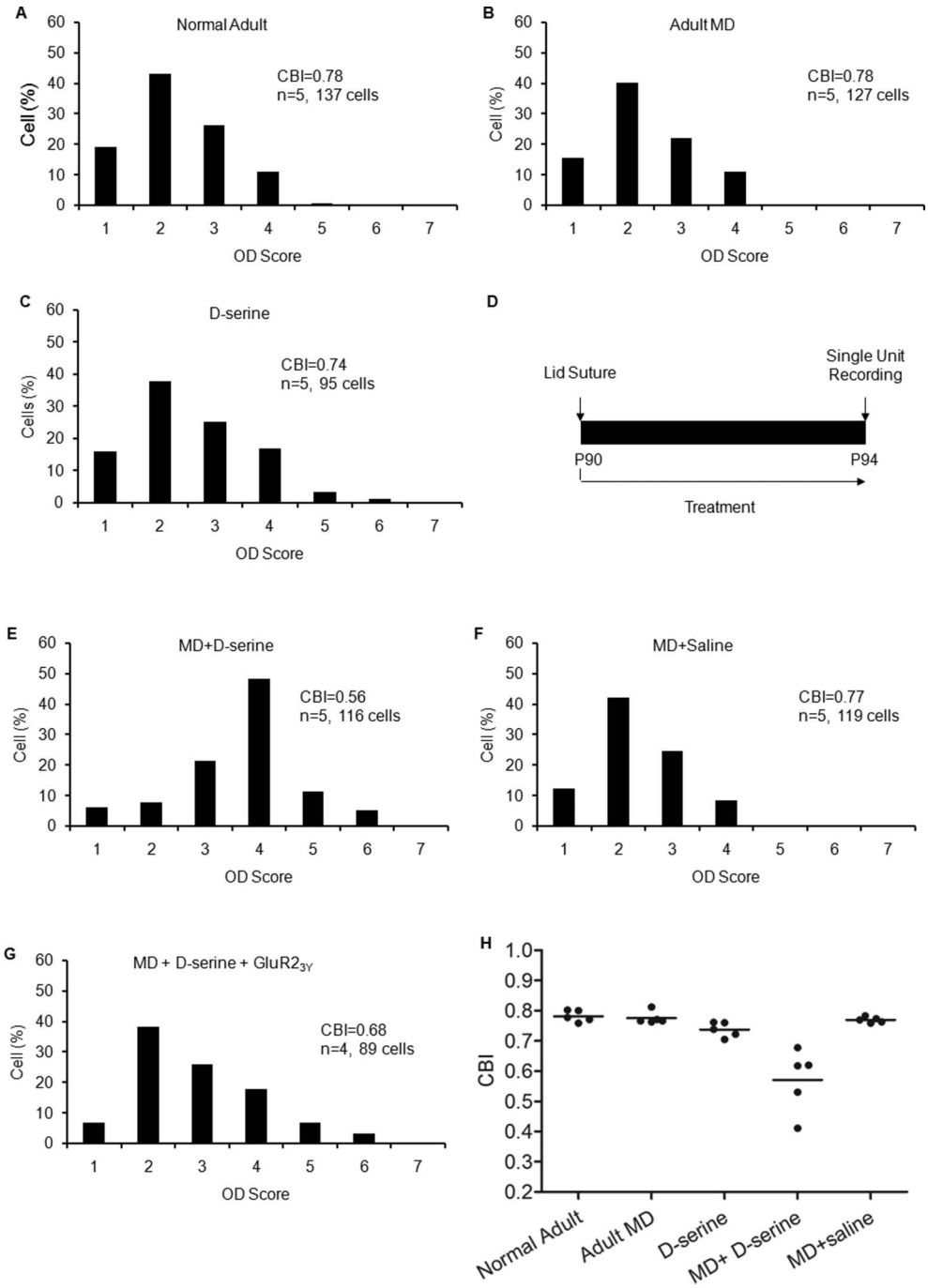

Figure 5 | D-serine enhances ocular dominance plasticity in adult mice. (A) Normal adult mice demonstrated a contralateral biased OD distribution (CBI=0.78±0.01, n=5). (B) single-unit recordings revealed no ocular dominance shift in monocularly deprived adult mice (CBI=0.78±0.01, n=5, p=0.64 compared to normal adult mice). (C) D-serine itself (600 mg/kg, bid, s.c.) did not induce an ocular dominance shift compared to untreated adult mice (CBI=0.74±0.01, n=5, p=0.03). (D) Experimental schedule for single-unit recording experiments. (E) D-serine (600 mg/kg, bid, s.c.) reinstated the ocular dominance shift after monocular deprivation in adult mice as is shown in the ocular dominance score distribution (CBI=0.56±0.05, n=5, p<0.01 compared to normal mice). (F) The saline treatment group did not show a corresponding shift in OD distribution (CBI=0.77±0.01, n=5, p=0.23 compared to normal mice). (G) GluR2$_{3Y}$ (10 nmol/g, i.p.) prevented the effect of D-serine (600 mg/kg, bid, s.c.) in monocularly deprived adult mice (CBI=0.68, n=4). (H) Data summary of the binocularity index results in the various conditions studied in adult mice.

cortical LTD and ocular dominance shift, we further tested its effectiveness in the amblyopic model, and found that D-serine treatment promoted the re-establishment of the contralaterally-dominated visual input in long-term monocular deprived mice (**Fig 7**). After long-term monocular deprivation starting at the beginning of the critical period (P23) and continuing until adulthood (P80), single-unit recordings revealed a significant ocular dominance shift towards the open eye (**Fig 7A,** CBI=0.50±0.05, n=5). Another group of long-term MD mice were reverse sutured at P80, and received treatment with either D-serine (600 mg/kg bid, s.c.) or saline for two weeks. The effectiveness of D-serine was assessed at P94, and the saline injection group served as controls (**Fig 7B**). Single unit recording data in the saline treatment group showed an ocular dominance shift towards the previously open eye (CBI=

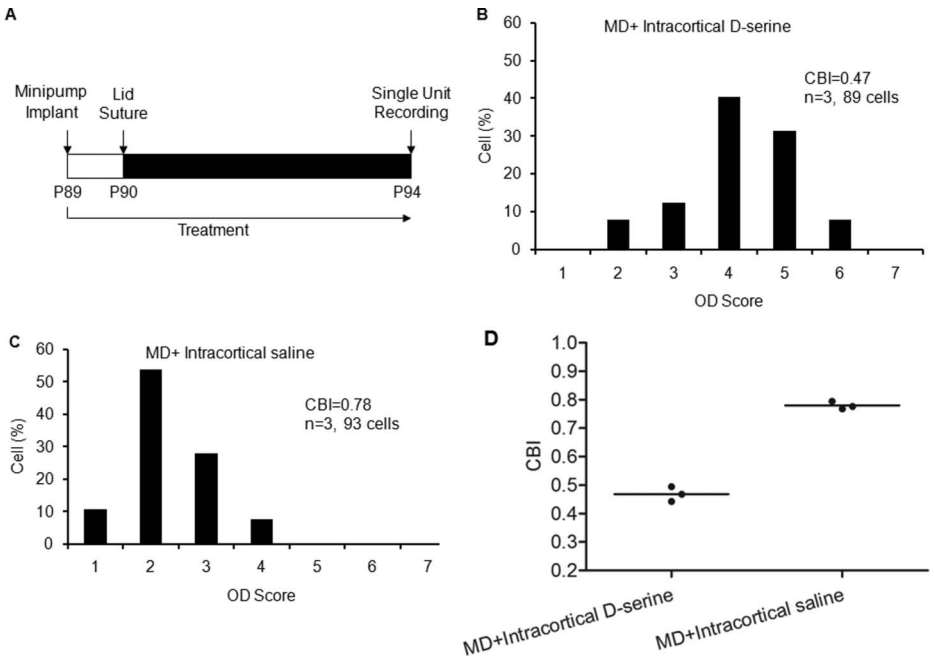

Figure 6 | D-serine enhances visual cortical plasticity in adult mice. (A) Experimental schedule for single-unit recording experiments in adult mice that received local infusion treatment. (B) D-serine-infused (50 μM) mice showed a significant ocular dominance shift (CBI=0.47±0.01, n=3, $p<0.01$ compared to normal mice). (C) Saline-infused mice demonstrated an OD distribution favoring the contralateral eye (CBI=0.78±0.01, n=3). (D) Data summary of the binocularity index in the D-serine intracortical infusion study.

0.51±0.03, n=4; **Fig 7D**), which is similar to the effects observed in the absence of reverse suture. The D-serine treatment group, on the other hand, showed much stronger visual input from the initially deprived eye (CBI=0.77±0.03, n=4; **Fig 7C, E**), indicating recovery of visual input in adulthood.

Discussion

Ocular dominance plasticity represents a well-studied example of experience-dependent modification of cortical function, and emerging evidence implicates several overlapping yet distinct mechanisms in the regulation of this plasticity. Among the well-studied phenomena involved in the regulation of visual cortex plasticity are the balance between excitation and inhibition in the cortex[27,28], the importance of regulation of axonal regenerative and sprouting capabilities via neurotropins and other regulatory molecules[6,29], a role for prior visual exposure[5], and processes such as LTP and LTD[30].

LTD and ocular dominance plasticity. Recent studies have closely associated LTD with learning and memory, and various pathological conditions[31]. Here we focused on the regulatory role of LTD in ocular dominance plasticity. LTD has been proposed as the cellular substrate for the loss of visual responsiveness following MD during the critical period[12,14,32,33]. With a strong LTD stimulation protocol, Jiang et al.[34] did not observe a decline of LTD that correlated with the closure of the critical period. Here, using a less saturating protocol, we were able to show that the magnitude of LTD induced in visual cortical slices declined during the critical period. LTD could be blocked in our juvenile visual cortical slices by applying a peptide that regulates AMPA receptor trafficking. This same peptide applied in juvenile mice prevented the ocular dominance shift after monocular deprivation.

Yoon et al.[14] reported similar findings using a different LTD blocking peptide G2CT, which disrupts the interaction of the GluR2 C-terminal tail with the AP2 clathrin adaptor complex and thereby interferes with the endocytosis of the AMPARs. G2CT blocked pair-pulse induced LTD in Layer IV spiny neurons of visual cortical slices and blocked MD *in vivo*. Using the same peptide as a LTD blocking agent, the same group also suggested that the molecular mechanisms of LTD in layer II/III were different from that of layer IV of the primary visual cortex, being independent of AMPA receptor endocytosis[35]. Our *in vitro* data, however, have shown that $GluR2_{3Y}$ inhibited layer II/III LTD. Several differences between the actions of the two different peptides might partially explain the discrepancies between our studies and that of Yoon et al.[14]. $GluR2_{3Y}$ specifically targets the tyrosine clusters on GluR2/3, thus avoiding the off-target effects that G2CT has on other endocytosis processes that are mediated by the AP2 adaptor. Different concentrations of peptide should also be considered when interpreting the data. We chose a higher concentration, because of the potential difficulty in achieving an effective concentration at the synapses, the primary site of action of these peptides.

NMDAR-regulated neurotransmission has been shown to play an important role in adult ocular dominance plasticity[10]. Here we showed that the administration of NMDAR co-agonist D-serine is effective in enhancing ocular dominance plasticity in adult mice. As an NMDAR co-agonist, D-serine operates in an activity-dependent manner. D-serine itself did not affect the baseline activity or responses in adult visual cortical slices, nor did it alter visual responses during our *in vivo* recordings. However, D-serine pre-incubation of visual cortical slices facilitated the induction of LTD in the presence of low-frequency stimulation. D-serine supplementation along with the manipulation of cortical activity by brief MD caused a significant OD shift that was otherwise absent in adult mice. This further supports the idea that exogenous co-agonist application in combination with the manipulation of cortical input can dynamically modulate NMDAR related plasticity. Moreover, D-serine specifically facilitated the induction of layer II/III LTD in adult visual cortical slices, without affecting that of LTP. Furthermore, the ability of D-serine to enhance the OD shift in adult mice was blocked by application of $GluR2_{3Y}$, again pointing specifically toward LTD as a key mechanism for enhanced adult plasticity.

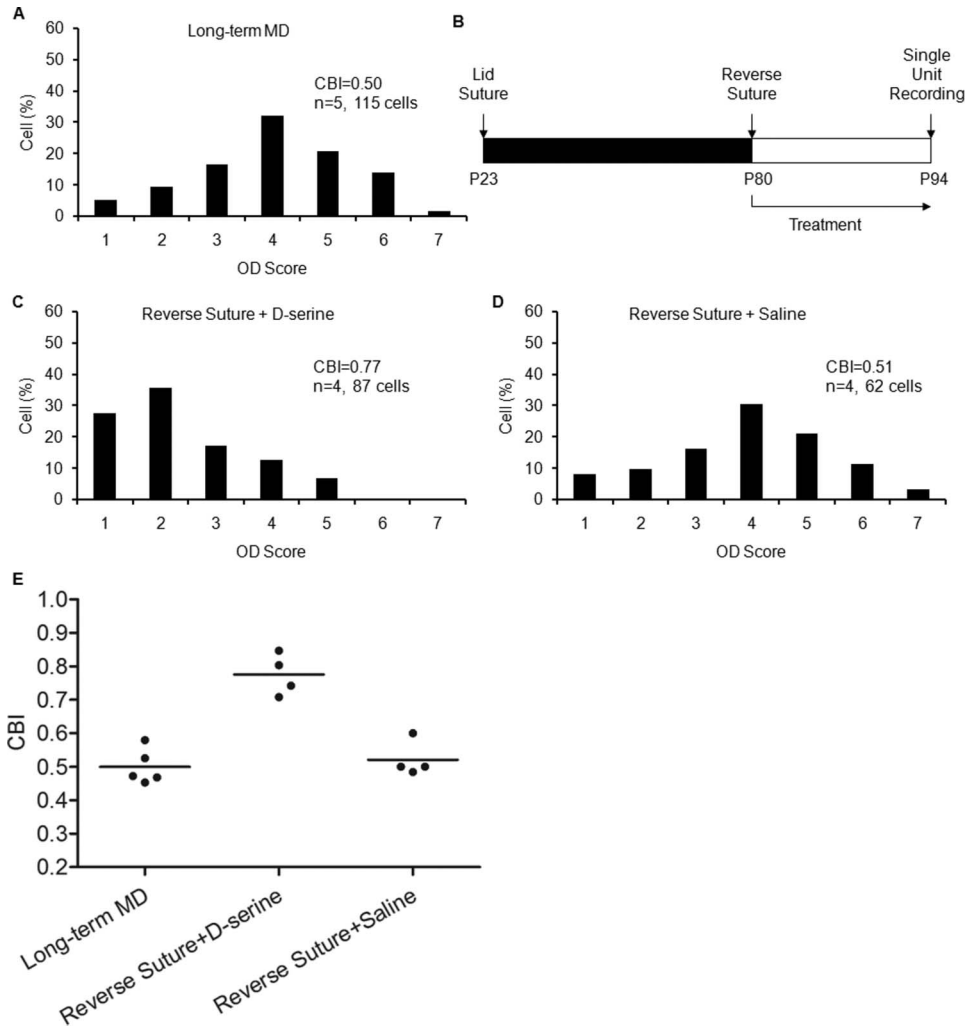

Figure 7 | D-serine promotes the restoration of normal binocularity in reverse sutured mice. (A) Long-term monocular deprivation results in an ocular dominance shift favoring the open eye into adulthood (CBI=0.50±0.05, n=5). (B) Experimental schedule for single-unit recording experiments in reverse sutured animals. (C) D-serine (600 mg/kg, bid, s.c.) promoted recovery of visual input from a previously deprived eye in reverse sutured mice (CBI=0.77±0.03, n=4). (D) Saline treated RS mice still showed dominance by the previously open eye (CBI=0.51±0.03, n=4). (E) CBI summary in long-term deprived mice, and in RS mice treated with D-serine or saline.

In our slice work, we focused on the study of layer IV to layer II/III LTD in ocular dominance plasticity. Previously, Trachtenberg et al.[36] reported loss of deprived-eye responsiveness in layer II/III prior to layer IV after brief monocular deprivation in cats. Studies in cats and ferrets suggest that organization of extragranular layers predicts the anatomical changes in the geniculocortical afferent during development[37–39]. Here we showed that blockade of layer II/III LTD prevented the OD shift in early postnatal life, and enhancement of layer II/III LTD in adult mice facilitated OD shift, suggesting that layer II/III LTD may play a special role in both juvenile and adult plasticity. At the same time, our results do not rule out a possible contribution of layer IV plasticity in either young or older mice.

As for the amblyopic model, recovery from long-term monocular deprivation initiated during the critical period does not occur readily in adulthood. After long-term MD spanning the critical period, reverse suture, or binocular experience alone is not potent[40–43]. A number of elegant studies have reported effective strategies to promote recovery of visual acuity after long-term monocular deprivation in rodents, such as dark exposure[44], the application of the antidepressant fluoxetine[45] and histone deacetylase inhibitors valproic acid and sodium butyrate[46]. Other strategies to enhance adult ocular dominance plasticity include degradation of chondroitin sulphate proteoglycans of the extracellular matrix by chondroitinase-ABC[29], mutation of the Nogo-66 receptor[6], the transplantation of inhibitory neurons[47], and the reduction of intracortical inhibition of mature visual cortex[48]. Current opinion favors the idea that these effects have been achieved either by resetting the excitatory–inhibitory (E–I) balance to mimic that occurring at critical period onset, or by removing molecular brakes that prevent structural changes after the critical period[27].

We combined reverse suture with D-serine treatment to restore contralaterally-biased visual input in long-term monocularly deprived mice. Reverse suture was used because it potentially allows a greater functional gain, as it shifts the competitive balance in favor of the initially deprived eye[49,50]. D-serine administration facilitated the shift in the cortical ocular dominance distribution after reverse suture, perhaps by promoting an LTD-like process altering inputs from the later-sutured eye. This idea is supported by our finding that the effect can be prevented by application of the LTD-blocking peptide $GluR2_{3Y}$. In adult visual cortical slices, D-serine specifically facilitated the induction of LTD while not affecting that of LTP.

Other than regulating NMDAR transmission, D-serine might conceivably have effects on the reinstatement of adult ocular dominance

plasticity through other mechanisms. A previous study suggested that D-serine might decrease GABAergic neurotransmission[51], which would contribute to a further increase of the E-I ratio, driving the visual system to a more immature state and making it easier for the recovery of function to occur in the reverse sutured mice. D-serine might also conceivably also have some effect on structural plasticity in addition to functional plasticity, since D-serine is mostly found in astrocytes that ensheathe NMDA-receptor-bearing neurons[52].

In summary, we prevented the ocular dominance shift with an LTD-blocking peptide in juvenile monocularly deprived mice, and restored visual cortical LTD and ocular dominance plasticity in adult mice with D-serine. As an endogenous NMDAR ligand with low toxicity, D-serine seems promising for aiding functional recovery from visual deficits.

Methods

All experiments were conducted in accordance with protocols approved by the Animal Care Center, University of British Columbia.

Mouse surgery. Male C57BL/6 mice (Charles River, Quebec, Canada) used for this study were maintained on a 12 h light/dark cycle with *ad libitum* access to food and water. Monocular deprivation induced by lid suture was performed under anesthesia induced by 3% isofluorane (Abbott, North Chicago, IL) in oxygen and maintained at 1.5%[24]. For adult short-term MD experiments, sutures were used to close the left eyelid around postnatal day 90 (P90). Long-term MD of the left eye started at the beginning of critical period around P23. Eyelid suture was maintained until P80 when the mice were reverse sutured. This was achieved by opening the left eyelid and closing the right eyelid. During the process, animals were checked daily to make sure that the eyelids were completely sealed. Mice whose eyelid fusion was incomplete and whose corneas showed signs of damage or cataract were excluded from the study.

Cortical Slice preparation. Coronal slices (each 400 μm thick) containing primary visual cortex were prepared for local field potential recordings. For the purposes of our study, we used mice of different ages, ranging from pre-critical period (before P21), critical period (P21–P43) and adulthood (around P90). Mice were anesthetized with urethane (5 mg/kg) and decapitated for brain extraction. The brains were prepared in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 5 KCl, 1 $MgSO_4$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 20 glucose. The ACSF was bubbled with 5 % CO_2/95 % O_2. A vibrating blade microtome (Leica, Germany) was used for cutting brain slices. Slices were placed in a submersion recording chamber with carbogenated ACSF and allowed to recover at 30 °C for ~1 h prior to recording.

***In vitro* electrophysiological recording.** Electrophysiological recordings were conducted in the chamber described above with continuous infusion of carbogenated ACSF at a rate of 1.5 ml/min at 30 °C. Extracellular field potentials were evoked via electrical stimulation through a concentric bipolar stimulating electrode (CBBRC75; FHC, Bowdoinham, ME) placed in the center of the cortical thickness that corresponded to layer IV, and recorded from layers II/III using glass electrodes (1 MΩ) filled with 1 M NaCl. Slices were evaluated for responsivity every 15 seconds with a constant current pulse of 100 μs duration and 100–200 μA of current, chosen to yield a half-maximal response. This field excitatory postsynaptic potential (fEPSP) was mainly mediated via AMPA receptors as it was completely abolished by the AMPAR antagonist DNQX (20 μM, Sigma). After a stable baseline was achieved, LTD was induced using a low frequency stimulation protocol (LFS) consisting of 900 stimuli at 1 Hz. To induce LTP, three to five episodes of theta-burst stimulation (TBS) were delivered at 10 sec intervals. TBS consists of ten stimulus trains delivered at 5 Hz. Each train consisted of four pulses at 100 Hz.

Whole-cell recordings of visual cortical neurons in brain slices were performed using the "blind" method with a MultiClamp 700B amplifier. Recording pipettes were filled with solution containing (mM) 132.5 Cs-gluconate, 17.5 CsCl, 2 $MgCl_2$, 0.5 EGTA, 10 HEPES, 4 ATP, and 5 QX-314, with pH adjusted to 7.2 by CsOH. For experiments to test the effect of the GluR2 peptide on LTD, $GluR2_{3Y}$ or $GluR2_{3A}$ (100 μg/ml) was also included in the recording pipettes. EPSCs were evoked similarly to fEPSPs and recorded while visual cortical neurons were voltage clamped at −60 mV. Synaptic responses were evoked at 0.05 Hz except during the induction of LTD, which was triggered by delivering low frequency stimulation (300 pulses at 1 Hz) while the recorded cell was voltage clamped at −45 mV. Induction of LTD was performed within 10 min of the establishment of the whole cell configuration to avoid washout of intracellular contents.

***In vivo* electrophysiological recording.** Electrophysiological recordings were performed under urethane anesthesia (50 mg/ kg, i.p., Sigma). Atropine (20 mg/kg s.c., Optopics) was injected to reduce secretions and parasympathetic effects of anesthetic agents. Dexamethasone (4 mg/kg s.c., American Reagent Laboratories) was administered to reduce cerebral edema. Mice were placed in a stereotaxic frame and a craniotomy was performed over the right side of the visual cortex. Agar was applied to the surface of the cortex to enhance recording stability and prevent desiccation. The eyelids were resected, and corneas were protected thereafter by frequent application of Ringer's solution. Body temperature was maintained at 37 °C using a homeostatic heating pad (Harvard). Heart rate was monitored continuously with electrocardiography (EKG) needles. Four to six sites (at least 100 μm apart) through the full thickness of the cortex were evaluated in each of four to six penetrations spaced evenly (at least 200 μm apart) crossing the binocular region (RF center azimuths <25 degrees from the vertical meridian) of area 17 to avoid sampling bias. In some cases large individual neuronal responses were isolated while other sites yielded multiunit activity. Sites were assigned to OD categories according to the seven-category scheme of Hubel and Wiesel[24]. OD histograms were constructed and contralateral bias index (CBI) scores were calculated for each mouse using the formula: $CBI = [(n_1-n_7)+(2/3)(n_2-n_6)+(1/3)(n_3-n_5)+N]/2N$, where N = total number of cells and n_x = number of cells with OD scores equal to x. The experimenters were blind to the treatment conditions of the mice.

Surgical implantation of minipumps. Osmotic minipumps (1007D, Alzet) were implanted for the purpose of local drug application. Mice were anesthetized with 3 % isofluorane in oxygen and mounted in a stereotaxic frame that allowed unobstructed vision. Ophthalmic lubricant was applied to protect the eyes, and body temperature was maintained at 37 °C with a heating pad. The minipumps were filled testing reagents and attached to 30 G stainless steel cannulae. Under aseptic conditions, a longitudinal incision was made in the scalp over the mid-sagittal sinus and the portions of the skull overlying occipital and frontal portions of the brain were cleaned and dried. To avoid any damage to the binocular zone of the primary visual cortex, the location of the infusion was centered in the cortical monocular zone, approximately 2 mm lateral to the midline and 1 mm rostral to lambda[53,54]. A small hole (D < 0.5 mm) was drilled through the skull above the occipital cortex contralateral to the deprived eye. The cannula was inserted to a depth of 1 mm under the surface of the skull and secured with cyanoacrylate adhesive (Alzet). The attached minipump was placed in a subcutaneous pocket at the nape of the neck. The scalp was closed over the implant, and the animal returned to its home cage. Minipump implantation was performed on the day prior to MD, and infusion continued for the duration of the MD, for a total of 5 days of infusion.

Peptide synthesis. $GluR2_{3Y}$ (YKEGYNVYG), $GluR2_{3A}$ (AKEGANVAG), Tat-$GluR2_{3Y}$ (YGRKKRRQRRRYKEGYNVYG), Tat-$GluR2_{3A}$ (YGRKKRRQRRRAKEGANVAG) and FITC-conjugated Tat-$GluR2_{3Y}$ peptides were synthesized by the Nucleic Acid and Peptide Service Centre at the University of British Columbia (Vancouver, Canada).

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Author contributions

Kaiyun Yang, Yu Tian Wang and Max Cynader wrote the main manuscript text. Kaiyun Yang, Wei Xiong, Guang Yang, and Luba Kojic prepared figures 1–7. All authors reviewed the manuscript.

Additional information

Competing financial interests: The authors declare no competing financial interests.

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