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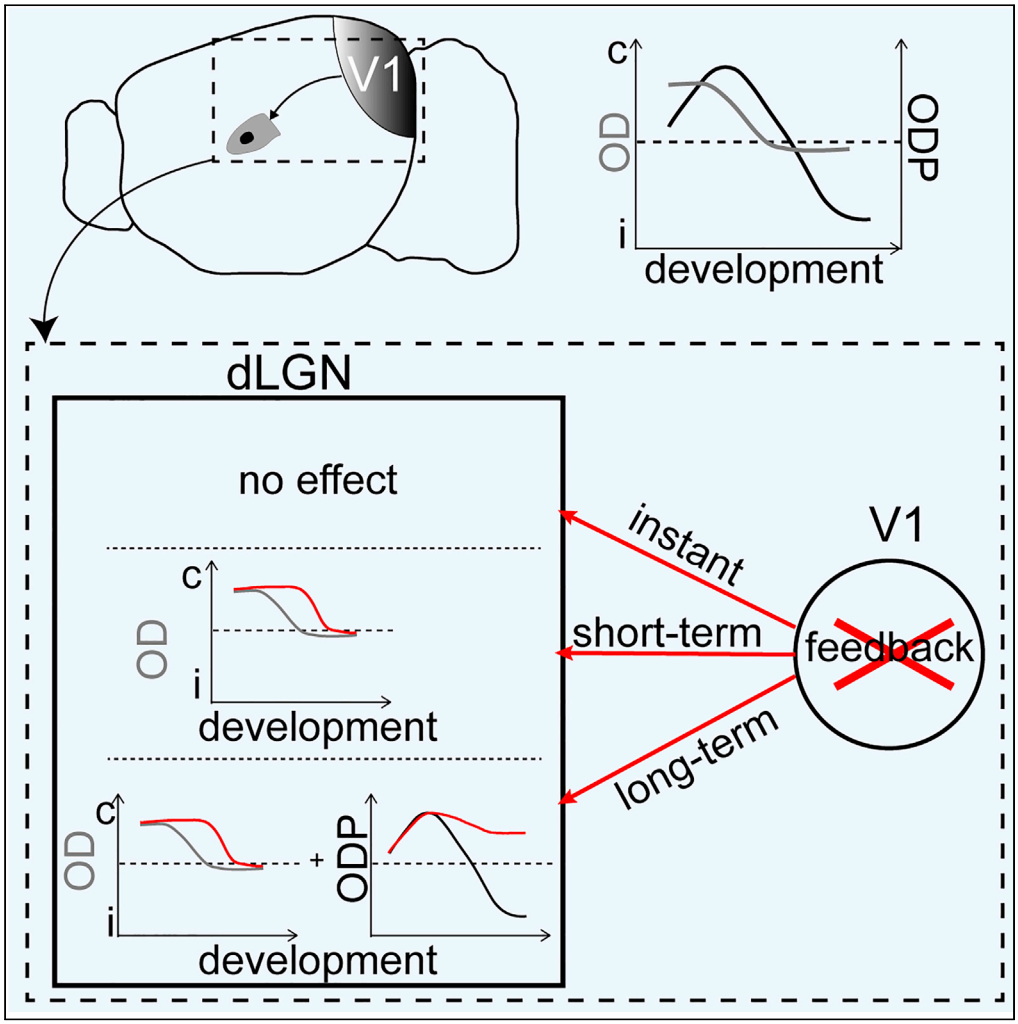

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Highlights

The OD of dLGN binocular neurons transformed from contralateral to equal bias at P24

The experience-dependent critical period for OD plasticity was from P14 to P30 in dLGN

Short-term V1 inactivation reversed the formation of OD bias in dLGN

Long-term V1 inactivation retained the development of dLGN to an immature stage

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Cortical feedback modulates distinct critical period development in mouse visual thalamus

Na Li,[1] Qiong Liu,[2] Yimu Zhang,[1] Zhongyi Yang,[3] Xuefeng Shi,[4,5,*] and Yu Gu[1,6,*]

SUMMARY

In primary visual cortex (V1), critical period for ocular dominance (OD) plasticity is a well-defined developmental stage to shape neuronal circuits based on visual experience. Recent studies showed that V1-like OD plasticity existed in mouse dorsal lateral geniculate nucleus (dLGN). It is still unclear what the exact time window is and how neural circuits contribute to OD plasticity in dLGN. Using *in vivo* electrophysiology, we defined a critical period for OD plasticity in dLGN from eye opening to puberty. There also existed an innate process of OD formation from contralateral to equal bias in dLGN binocular neurons. Instant V1 inactivation with muscimol had no effect on OD bias or plasticity. Short-term V1 inactivation with N-methyl-d-aspartate reversed the formation of equal OD bias, while long-term V1 inactivation retained dLGN development to an immature stage.

INTRODUCTION

Critical period is defined as a hyperplastic stage during early development, which enables dramatic neuronal rewiring and rearrangement for the proper functional maturation. In primary visual cortex (V1), experience-dependent critical period for ocular dominance (OD) plasticity was required for the normal development of binocular visual circuits.[1,2] That is to say, the disruption of binocular vision, such as monocular depriving (MD) of the contralateral eye during the critical period, will cause normal contralateral biased OD shift to the non-deprived eye and abnormal development of visual functions, such as amblyopia.[3,4] It was generally believed that the visual information from each eye was firstly converged onto a single binocular neuron at layer **IV** of V1.[5] Thus, neurons in the dorsal lateral geniculate nucleus (dLGN) were thought to receive exclusively contralateral or ipsilateral retinal input and displayed no classic OD plasticity.[6–8] However, recent studies using single-cell-initiated transsynaptic tracing revealed that a single neuron in dLGN could receive inputs from both eyes.[9] *In vivo* electrophysiological experiments in marmosets and mice have also shown that single dLGN neuron could respond to both contralateral and ipsilateral stimuli.[10–12] These anatomical and functional evidences suggested indeed binocular integration in dLGN. In addition, emerging evidences indicated that there existed OD plasticity in dLGN, similar to V1. *In vivo* electrophysiology in juvenile mice showed a portion of dLGN neurons displayed OD shift after 7-day MD.[13] Another study reported that 8-day MD induced OD shift in adult mice using two-photon imaging.[14] However, the duration of MD they performed was longer than that generally used in V1 critical period, and they did not rule out the effect of V1 feedback, which might directly drive the OD shift in dLGN. In contrast to the above studies, Carey and Karim reported that long-term MD in juveniles led to a decrease of the number of binocular responsive neurons in dLGN, not OD shift.[15] Thus, it remains unclear whether V1-like OD plasticity exists in dLGN.

One remaining question concerning the influence of MD on dLGN development is the exact mechanism that drives the OD shift. Previous studies demonstrated that besides retinal inputs, dLGN also receives V1 feedback of layer **VI** neurons through direct excitatory projection as well as indirect inhibitory projection via thalamic reticular nucleus (TRN).[16–18] Accumulating evidences suggest that V1 feedback contributes to the refinement of dLGN circuits,[19,20] and with disrupted corticothalamic projection in juveniles, the inputs from the retina were also affected.[21,22] In addition, V1 feedback can influence dLGN gain modulation, sharpen or shift dLGN receptive fields, and change dLGN spiking mode, timing, and precision.[23–26] Therefore, V1 feedback may play an instrumental role in the development of dLGN. It is interesting to ask whether V1 feedback contributes to the changes of binocularity in dLGN with visual deprivation.

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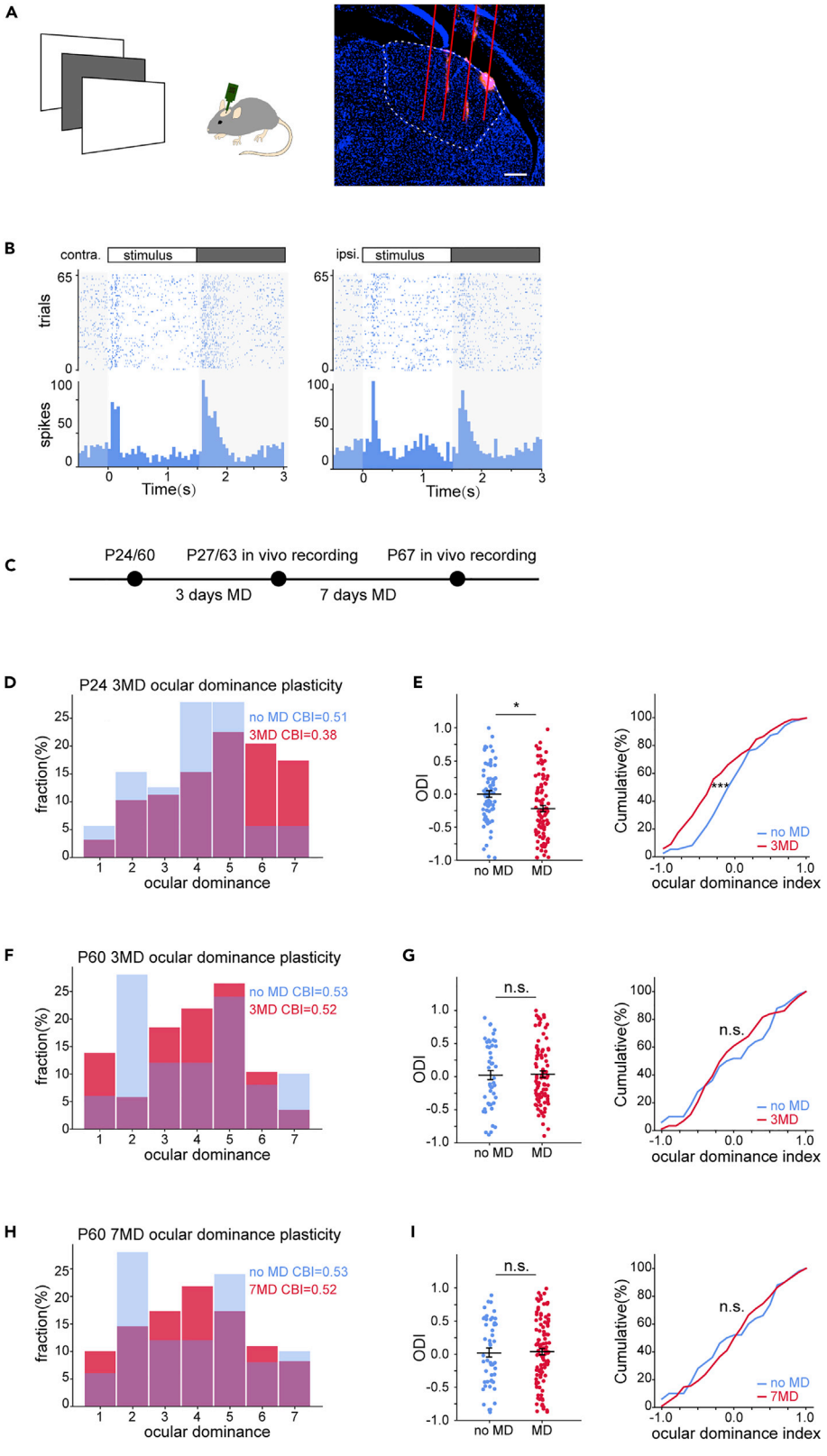
A
B
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Time(s)
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P24/60
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P67 in vivo recording
3 days MD
7 days MD
D
P24 3MD ocular dominance plasticity
no MD CBI=0.51
3MD CBI=0.38
fraction(%)
ocular dominance
E
ODI
no MD
MD
Cumulative(%)
ocular dominance index
F
P60 3MD ocular dominance plasticity
no MD CBI=0.53
3MD CBI=0.52
G
n.s.
H
P60 7MD ocular dominance plasticity
no MD CBI=0.53
7MD CBI=0.52
I

Figure 1. 3-day MD induced OD shift of on response in juvenile dLGN binocular neurons with on-off stimuli

(A) Left, schematic setup to measure OD. *In vivo* extracellular electrophysiology was performed in dLGN of anesthetic mice with on-off full-screen visual stimulus. Right, a representative image showing 4 × 8 multichannel recording probe in the mouse dLGN. Tracks of the four-shank electrode (red line) are visible owing to deposition of DiI applied to the probe prior to insertion. White dotted line represents the dLGN border. Scale bar, 200 μm.

(B) Examples raster plots of dLGN single-unit on and off responses to on (full-screen white) and off (full-screen gray) stimuli performed to the contralateral (left) and ipsilateral eye (right) (top: the type of stimuli; middle: raster plots of spikes; bottom: spike numbers of each bins).

(C) The timeline of MD and *in vivo* recording at different age.

(D, F, and H) The fraction of OD distributions for binocular response in dLGN neurons of no MD (blue) *vs.* MD (red) mice. (D) The OD distribution of P24 with 3-day MD; (F) The OD distribution of P60 with 3-day MD; (H) The OD distribution of P60 with 7-day MD.

(E, G, and I). The scatter plot (left) and the cumulative distribution (right) of ocular dominance index of visual response in dLGN binocular neurons of no MD (blue) *vs.* MD (red) mice. Same data as shown in (D, F, and H). Each point represents the relative response strength for two eyes of a single dLGN neuron. (E) comparing no MD *vs.* 3-day MD at P24 ($p = 0.0392$, Nested t test, two-sided; $p = 0.00045$, Kolmogorov-Smirnov test; no MD = 72 cells from 4 mice, MD = 98 cells from 10 mice); (G) comparing no MD *vs.* 3-days MD at P60 ($p = 0.912$, Nested t test, two-sided; $p = 0.373$, Kolmogorov-Smirnov test; no MD = 50 cells from 6 mice, MD = 87 cells from 6 mice); (I) comparing no MD *vs.* 7-day MD at P60 ($p = 0.893$, Nested t test, two-sided; $p = 0.764$, Kolmogorov-Smirnov test; no MD = 50 cells from 6 mice, MD = 110 cells from 6 mice). Error bar indicates mean ± SEM. n.s., $p > 0.05$,*$p \leq 0.05$, **$p < 0.01$ and ***$p < 0.001$.

Here, we investigated the OD plasticity and its origin in mouse dLGN, using *in vivo* electrophysiology. We found that there existed two different developmental processes in dLGN binocular neurons, the formation of equal OD bias and the critical period for OD plasticity. In addition, pharmacological blockade of V1 feedback during critical period for instant, short-term or long-term durations distinctly modulates the development of dLGN. These data showed V1 feedback was required for the proper formation of OD bias, and lacking V1 feedback at early age would pause the normal development of dLGN. Taken together, our findings suggested that the normal dLGN development had distinct processes and required proper cortical feedback.

RESULTS

3-day MD was sufficient to induce OD shift of binocular responses in juvenile dLGN

Previous studies have reported that some dLGN neurons underwent OD shift after short-term MD in juveniles[13] and long-term MD in adulthood.[14] To confirm whether there exists OD shift in mouse dLGN, we performed full-screen white (1.5s) and black (1.5s) on-off stimulus to the contralateral and ipsilateral eye and used four-shank silicon probe to record the responses of dLGN neurons (Figure 1A). Consistent with previous studies,[13,27] majority of the recorded neurons had both on (respond to stimuli) and off responses (respond after stimuli) (Figure 1B). Only the binocular neurons which responded to both contralateral and ipsilateral stimulus were included to measure the OD in the following experiments. Interestingly, the response of normal dLGN neurons at P24 showed neither contralateral nor ipsilateral bias (equal OD, contralateral bias index (CBI) close to 0.5, Figure 1D), unlike the strong contralateral bias in V1.[28,29] But after 3-day MD of the contralateral eye, the OD was significantly shifted to the ipsilateral eye (Figures 1C–1E). It is interesting to ask whether the effects of MD on dLGN neurons exist throughout the whole lifespan or only at the early age. Thus, short-term and long-term MD were performed in adult mice at P60. 3-day or even 7-day MD could not change the equal OD of dLGN response (Figures 1C and 1F–1I). Considering 7-day MD is sufficient to induce OD shift in adult V1, these data suggested that the sensitivity to MD was restricted to juveniles in dLGN, and the adult dLGN might be even less plastic than adult V1. Our findings revealed that 3-day MD was sufficient to induce OD shift of binocular responses in juvenile dLGN with on-off stimulus, which was not seen in adults.

Distinct critical period for OD bias and plasticity in dLGN binocular neurons

It remains unclear whether there is a critical period in dLGN which is sensitive to the unbalanced visual experience in early development, similar to V1. To test this, we measured visual responses of the two eyes in dLGN using *in vivo* electrophysiology in mice that were normally reared or MD for 3 days at P14 (eye open), P19, and P30 (Figure 2A). Interestingly, at P14 and P19, the early stage of visual development in mice, the normal mice showed strong contralateral bias that was different from the equal OD bias at P24 (Figures 2B and 2C vs. Figure 1D). But after MD, the ocular dominance index (ODI) reduced significantly and OD shifted to the open eye at P19 and changed slightly at P14 (Figures 2B and 2C). Comparing the firing rates of the two groups, we found that the OD shift was mainly contributed from a decrease in contralateral

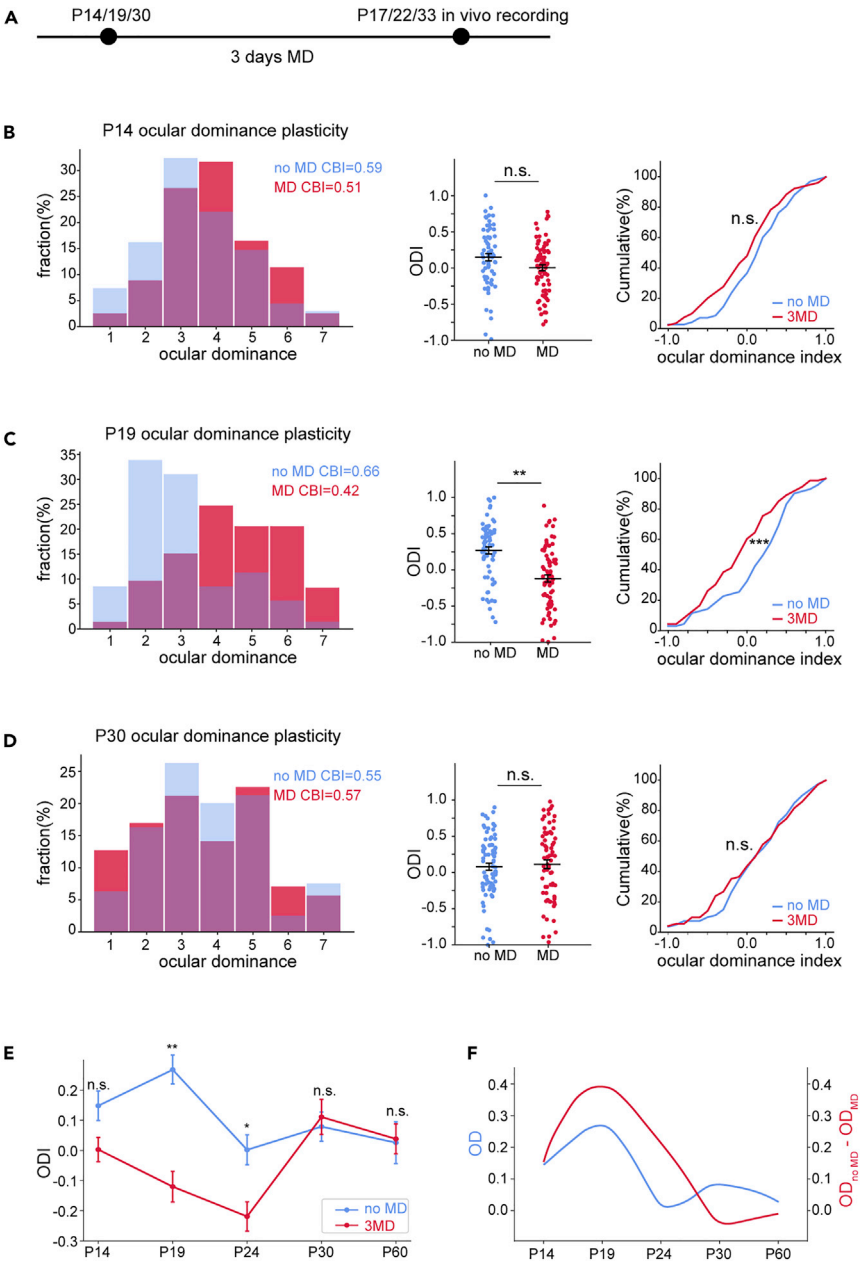


Figure 2. The OD bias and OD plasticity of mouse dLGN binocular neurons changed with development

(A) The timeline of MD and *in vivo* recording at different ages.

(B–D) The fraction of OD distributions (left) and scatter plot (middle) and the cumulative distribution (right) of ocular dominance index for binocular response in dLGN neurons of no MD (blue) *vs.* MD (red) mice at different ages. (B) the OD change of P14 mice ($p = 0.105$, Nested t test, two-sided; $p = 0.053$, Kolmogorov-Smirnov test; no MD = 68 cells from 6 mice, MD = 79 cells from 6 mice); (C) the OD shift of P19 mice (P19: $p = 0.005$, Nested t test, two-sided; $p < 10^{-7}$, Kolmogorov-Smirnov test; no MD = 71 cells from 7 mice, MD = 73 cells from 5 mice); (D) the OD distribution of P30 mice ($p = 0.743$, Nested t test, two-sided; $p = 0.620$, Kolmogorov-Smirnov test; no MD = 80 cells from 9 mice, MD = 71 cells from 7 mice). n.s., $p > 0.05$,*$p \leq 0.05$, **$p < 0.01$ and ***$p < 0.001$.

(E) The summary of the OD index for binocular response in dLGN neurons of no MD (blue) *vs.* MD (red) mice at all ages. Same data as shown in Figures 1D and 2B–2D. Error bar indicates mean ± SEM.

(F) The smooth curves to show the trend of OD bias change (blue, namely the mean OD index, 0 represented equal OD bias and positive number represented contralateral bias), and the level of OD plasticity (red, namely the mean ODI difference between no MD and MD mice) during early postnatal development. Same data as shown in figure (E).

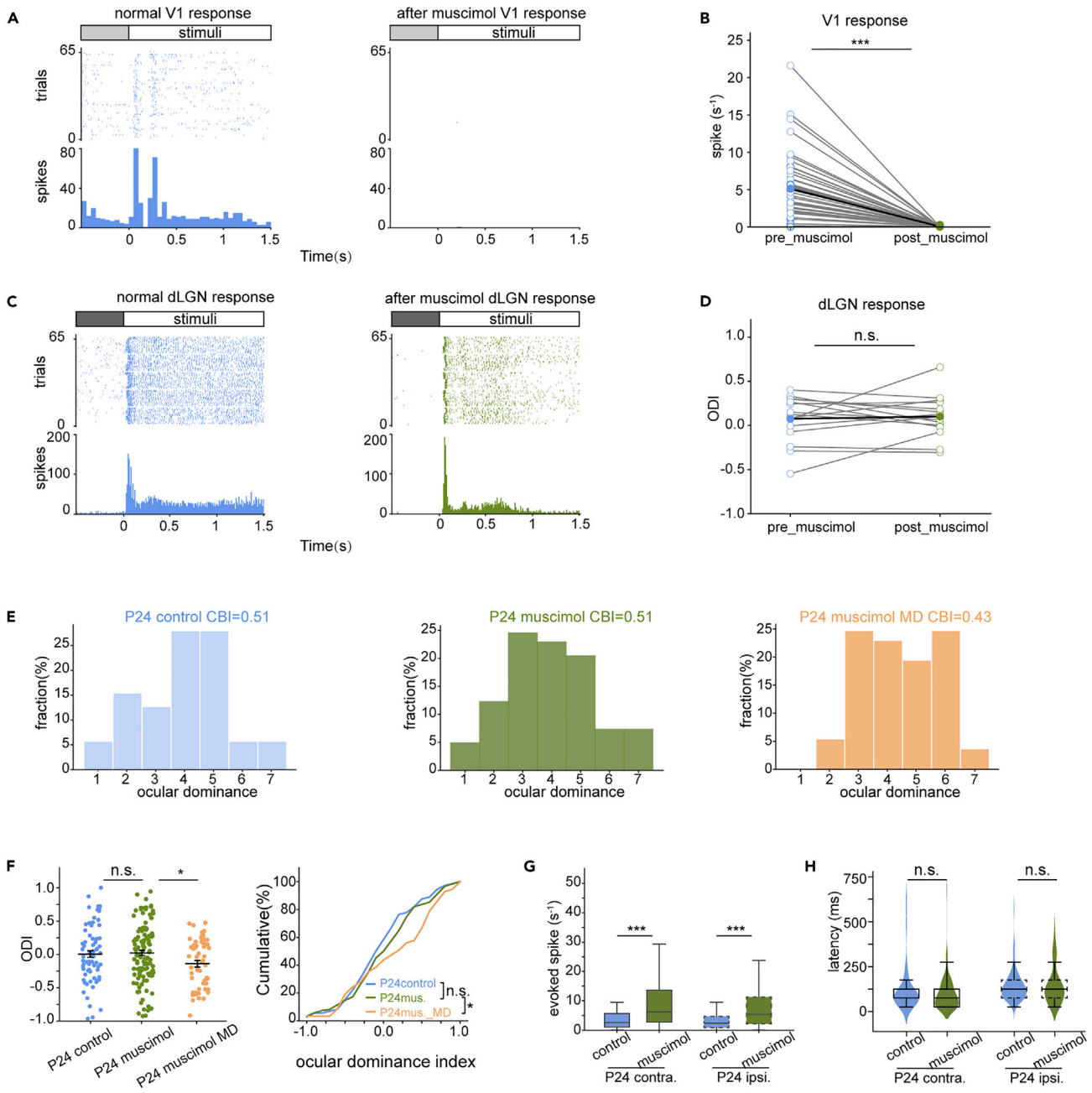


Figure 3. The effect of instant V1 inactivation on OD bias and plasticity in dLGN binocular neurons

(A) An example of V1 single-unit response before (left) and after (right) muscimol application.

(B) The spike activity of single V1 neuron before and after muscimol application ($p < 10^{-13}$, Wilcoxon signed-rank test, two-side; n = 47 cells from 2 mice.). Lines connect individual neurons (open circle); filled circle and black line indicates the change magnitude in units of mean value between two groups.

(C) An example of dLGN contralateral response before (left) and after (right) muscimol application.

(D) The ocular dominance index of single dLGN neuron before and after muscimol application (p = 0.978, Wilcoxon signed-rank test, two-side; n = 15 cells from 2 mice.). Lines connect individual neurons (open circle); filled circle and black line indicates the change magnitude in units of mean value between two groups.

(E) The fraction of OD distributions for dLGN neurons of control (blue), muscimol (green), and muscimol+MD (orange) mice at P24.

(F) Scatter plot (left) and the cumulative distribution (right) of OD index of three experimental groups as shown in B. (p= 0.100, Nested one way ANOVA, control *vs.* muscimol: p = 0.812; muscimol *vs.* muscimol MD: p = 0.040. Kolmogorov-Smirnov test, control *vs.* muscimol: p = 0.779; muscimol *vs.* muscimol MD: p = 0.047; control = 72 cells from 4 mice, muscimol = 122 cells from 9 mice, muscimol MD = 57 cells from 8 mice). Error bar indicates mean ± SEM.

Figure 3. *Continued*

(G) Box plots of contralateral (solid line) and ipsilateral (dotted line) responses in dLGN binocular neurons of no MD control *vs.* muscimol mice at P24 (contralateral: control *vs.* muscimol $p < 10^{-6}$, control = 66 cells from 4 mice, muscimol = 119 cells from 9 mice; ipsilateral: control *vs.* muscimol $p < 10^{-7}$, control = 74 cells from 4 mice, muscimol = 116 cells from 9 mice. Wilcoxon rank-sum test, two side). In each box plot, the central mark indicates the median, bottom and top edges indicate the 25th and 75th percentiles, and the most extreme data points were considered outliers (1.5 times the interquartile range). (H) The violin and overlaid box plots of contralateral and ipsilateral response latency in dLGN neurons of control *vs.* muscimol mice (contralateral: control *vs.* muscimol $p = 0.003$, control = 84 cells from 4 mice, muscimol = 129 cells from 9 mice; ipsilateral: control *vs.* muscimol $p = 0.138$, control = 87 cells from 4 mice, muscimol = 117 cells from 9 mice. Kolmogorov-Smirnov test). n.s., $p > 0.05$,*$p \leq 0.05$, **$p < 0.01$ and ***$p < 0.001$.

response, similar to V1 (Figures S2A–S2K). In a similar experiment at P30, we found that the contralateral and ipsilateral responses tended to be equal in normal dLGN, while MD could not induce OD shift at this age (Figure 2D). Interestingly, the change of firing rates during normal development, especially the contralateral response, was similar to the change of OD in normal dLGN development (Figures S2A–S2K). Through electrode reconstruction, we confirmed that the change of OD bias between distinct ages was not due to the difference of dLGN recording sites (Figure S1).

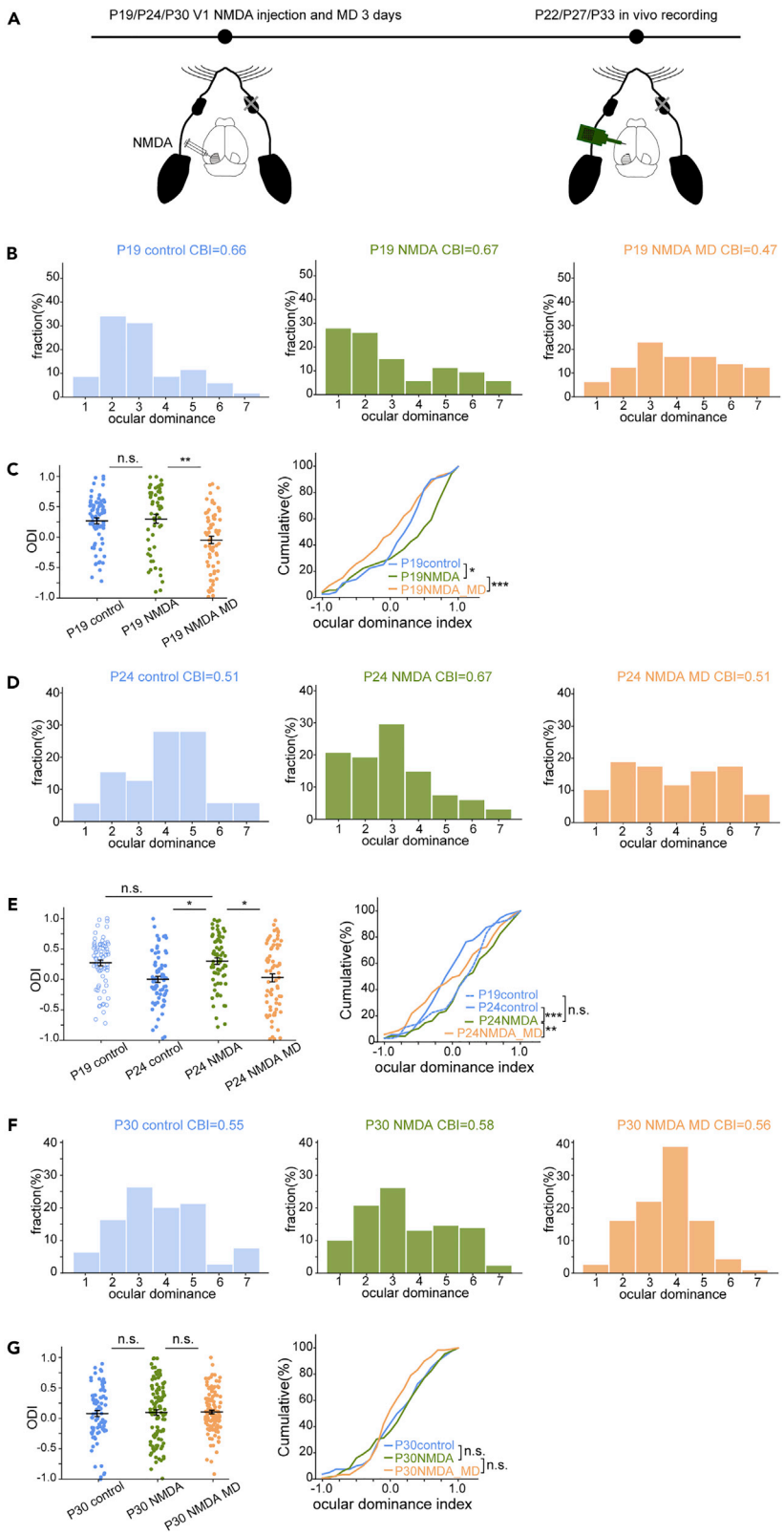
Thus, we proposed there were two types of developmental processes of visual functions which reflected the relative contralateral and ipsilateral responsive strength in dLGN binocular neurons, and they had different time windows (Figures 2E and 2F). One was the change of OD bias during the normal development. At early ages (P14-P19), the OD showed strong contralateral bias. Then around P24, the OD switched to equal, which was retained into adulthood. Though the evoked spike rates of both contralateral and ipsilateral response reduced, the change of OD could be mainly caused by a developmental decrease of the contralateral responses (Figure S2K). The other one was OD shift induced by MD, namely experience-dependent OD plasticity. After eye opening at P14, the OD plasticity emerged and reached its peak at P19. MD mainly led to the decrease of contralateral responses during this period (Figure S2K). After P30, the critical period closed and OD plasticity declined, and there was no change of firing between normal and MD groups. These suggest that the experience-dependent critical period for OD plasticity in dLGN is from P14 to P30, which is earlier than that of V1 (typically P21-35[5,30]). Throughout the whole ages, the fraction of responsive neurons showed no obvious changes (Figure S2L), and MD did not cause neuronal death in dLGN (Figure S3).

Instant V1 inactivation had no effects on OD bias and plasticity in dLGN binocular neurons

Previous studies demonstrated that there existed remodeling of the retinogeniculate projections after receiving visual information.[31,32] Moreover, V1 feedback contributed to the refinement of retinogeniculate projections,[21] and blocking corticothalamic projection in juveniles could lead to abnormal development of dLGN.[16,21,22] In addition, dLGN responses could be strongly modulated by V1 feedback, through either direct corticothalamic projection or indirect projection via TRN.[23–26] That raises the question whether V1 feedback contributes to the different developmental stages between OD formation and OD plasticity. It is possible that the OD shift in dLGN we observed was completely inherited from the V1 feedback, without further remodeling of the local circuit in dLGN. To test this, we instantly inactivated V1 activity ipsilateral to the recorded dLGN by topically applying muscimol, a $GABA_A$ receptor agonist, to the brain surface without dura mater. Similar to previous reports,[10,14] muscimol completely abolished the responses in both superficial and deep layers in V1 within 3 hours, which could block the instant V1 feedback to dLGN but not silence the response of dLGN (Figures 3A–3C). The electrophysiological results showed that the equal OD bias and robust OD shift in dLGN remained unaffected after V1 inactivation at P24 (Figures 3D–3F). Interestingly, the instant V1 inactivation led to an increase of firing rates of both contralateral and ipsilateral responses (Figure 3G), which was similar to a recent result with optogenetic inhibition of the layer VI of V1.[33] In addition, muscimol did not affect the latency of both contralateral and ipsilateral responses in dLGN (Figure 3H). These data indicated that V1 might provide a real-time and overall inhibitory effect on dLGN via either TRN or local dLGN inhibitory neurons.

Short-term V1 inactivation reversed the formation of OD bias in dLGN binocular neurons

Although instant V1 inactivation had no effect on dLGN OD bias and plasticity, there still exists possibility that longer duration of V1 inactivation could have an effect during the critical period development. To examine this, we utilized pharmacological method to lesion V1 by injecting N-methyl-d-aspartate (NMDA, an excitatory neurotransmitter, can produce excitotoxic injury to kill cells[22,34]) into the left V1, ipsilateral to the recorded dLGN, and immediately MD the right eye for 3 days (Figures 4A and S4A–S4D). When we injected NMDA at the peak of OD plasticity of dLGN (P19), the OD remained contralateral


A
P19/P24/P30 V1 NMDA injection and MD 3 days
P22/P27/P33 in vivo recording
NMDA
B
P19 control CBI=0.66
P19 NMDA CBI=0.67
P19 NMDA MD CBI=0.47
fraction(%)
ocular dominance
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P19 control
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P24 control CBI=0.51
P24 NMDA CBI=0.67
P24 NMDA MD CBI=0.51
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P19 control
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P30 control
P30 NMDA
P30 NMDA MD
P30control
P30NMDA
P30NMDA_MD
n.s.
n.s.

Figure 4. Short-term V1 inactivation reversed the development of OD bias in dLGN binocular neurons at P24

(A) Experimental timeline (upper) and schematic of dLGN recording with stereotaxic NMDA injection to inactivate V1 (lower). After NMDA injection, MD was performed on the right eye immediately, and dLGN response was recorded 3 days later.

(B, D, and F) The fraction of OD distributions for dLGN neurons of control (blue), NMDA (green), and NMDA+MD (orange) mice in P19, P24, and P30.

(C, E, and G) Scatter plot (left) and the cumulative distribution (right) of OD index of three experimental groups in three ages as shown in (B, D, and F). (C) The change after short-term V1 inactivation at P19 ($p = 0.003$, Nested one way ANOVA; the change of OD bias, left and middle, $p = 0.788$, Dunn-corrected post hoc test; $p = 0.012$, Kolmogorov-Smirnov test; control = 71 cells from 7 mice, NMDA = 54 cells from 7 mice; the change of OD shift, middle and right, $p = 0.003$, Dunn-corrected post hoc test; $p = 0.0001$, Kolmogorov-Smirnov test; NMDA = 54 cells from 7 mice, NMDA MD = 66 cells from 7 mice); (E) The change of P24 ($p = 0.008$, Nested one way ANOVA; the change of OD bias, P24 control *vs.* P24 NMDA: $p = 0.033$, Dunn-corrected post hoc test; $p = 0.0001$, Kolmogorov-Smirnov test; control = 72 cells from 4 mice, NMDA = 68 cells from 7 mice; the change of OD shift, P24 NMDA *vs.* P24 NMDA_MD: $p = 0.012$, Dunn-corrected post hoc test; $p = 0.004$, Kolmogorov-Smirnov test; NMDA = 68 cells from 7 mice, NMDA MD = 69 cells from 6 mice; P19 control *vs.* P24 NMDA: $p = 0.952$, Dunn-corrected post hoc test; $p = 0.114$, Kolmogorov-Smirnov test); (G) The change of P30 ($p = 0.990$, Nested one way ANOVA; left and middle, $p = 0.997$, Dunn-corrected post hoc test; $p = 0.391$, Kolmogorov-Smirnov test; control = 80 cells from 9 mice, NMDA = 108 cells from 8 mice; middle and right, $p = 0.986$, Dunn-corrected post hoc test; $p = 0.054$, Kolmogorov-Smirnov test; NMDA = 108 cells from 8 mice, NMDA MD = 119 cells from 6 mice). Error bar indicates mean ± SEM. n.s., $p > 0.05$,*$p \leq 0.05$, **$p < 0.01$ and ***$p < 0.001$.

bias, and 3-day MD could still shift OD to the ipsilateral eye, similar to normal controls (Figures 4B and 4C). At a later age (P24), however, 3-day V1 inactivation changed the OD from equal to contralateral bias (Figures 4D and 4E), similar to the OD bias at an early age, which was mainly due to the increase of contralateral responses (Figure S4H). Though the morphology was normal, the fraction of binocular neurons was decreased, and the neurons responding to the contralateral eye increased significantly (Figures S4E and S4F). Interestingly, the OD plasticity remained unaffected between control and NMDA-treated mice at P24 (Figures 4D and 4E). In addition, at P30 (when the critical period closes), 3-day NMDA inactivation of V1 did not affect either OD bias or plasticity (Figures 4F and 4G). However, the change of evoked response properties was similar to instant inactivation with muscimol, such as increasing firing rates (Figures S4M and S4N vs. Figures 3G and 3H). Together, these results demonstrated that 3-day V1 inactivation reversed the formation of OD bias at P24 especially through the contralateral response modulation, but not OD plasticity. Either before or after P24, short-term V1 inactivation had no effect on dLGN development. That is to say, at the specific developmental stage (P24) in the normal development, V1 feedback is required for the decline of the contralateral responses in dLGN binocular neurons to promote the formation of equal OD bias.

Long-term V1 inactivation retained the development of dLGN to an immature stage

Previous data showed that V1 feedback contributed to the refinement of retinogeniculate connection which correlated to OD plasticity.[21] Although we showed that short-term V1 inactivation only affected the formation of OD bias at P24 (Figure 4), it is interesting to ask whether the V1 feedback played a more extensive role for the development of OD plasticity in dLGN critical period. To fully assess this question, we inactivated V1 with NMDA before the onset of robust visual responses occurred during development to totally exclude V1 influence and recorded the responses of dLGN binocular neurons after the critical period at P30 (Figure 5A). We found that the OD remained contralateral bias like P19, the time point when we inactivated V1, different from the equal OD bias in normal control at P30, the time point of recording (Figures 5B and 5C). The firing rate was also similar to P19 (Figure S5D). And the responsive fraction with long-term NMDA treatment was close to P19, not P30 (Figure S5B). Interestingly, 3-day MD at P30 in the long-term V1-inactivated mice could still shift the OD to the non-deprived eye (Figures 5B and 5C), which was resulted from the decrease of contralateral responses, similar to P19 (Figure S5E). Furthermore, the loss of V1 feedback from early age led to abnormal morphological development of dLGN, with a shrunken size and a higher cell density (Figure S5A). The results showed that lacking V1 feedback retained the development of dLGN to an immature stage of both OD bias and OD plasticity and implied that V1 feedback played an important role in the critical period development of dLGN, consistent with previous studies.[21,22]

DISCUSSION

Using *in vivo* extracellular electrophysiological recordings and pharmacological manipulations, we showed the dLGN development had distinct processes and required cortical feedback. Namely, the OD transforms

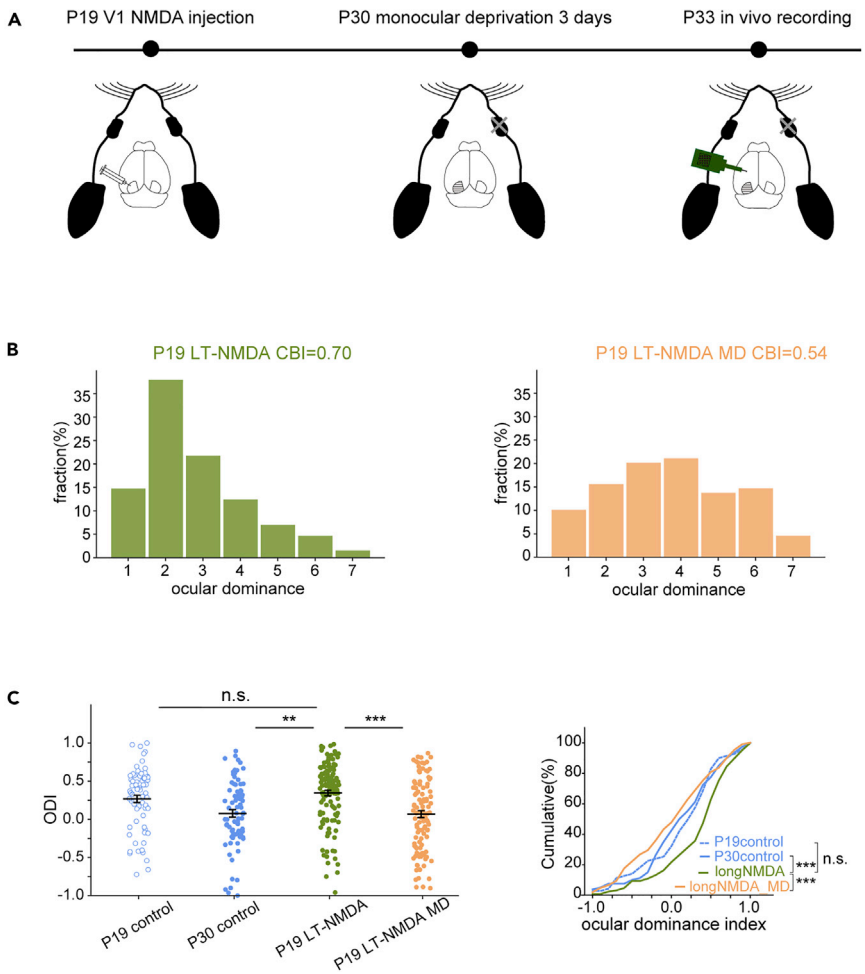

Figure 5. Long-term V1 inactivation retained dLGN development at an immature stage

(A) Experimental timeline (upper) and schematic setup (lower). NMDA was injected into V1 at P19 for long-term V1 inactivation. After NMDA injection, MD was performed on the right eye at P30, and dLGN response was recorded 3 days later.

(B) The fraction of OD distributions for dLGN neurons of long-term NMDA (green) and LT-NMDA+MD (orange) mice.

(C) Scatter plot (left) and the cumulative distribution (right) of OD index of four experimental groups as shown in B and Figure 4B (left), F(left). Long-term NMDA reversed OD bias (The difference between the first three, $p = 0.0002$, Nested one way ANOVA; P19 control *vs.* P19 LT-NMDA: $p = 0.530$; P30 control *vs.* P19 LT-NMDA: $p = 0.002$, Dunn-corrected post hoc test. P19 control *vs.* P19 LT-NMDA: $p = 0.301$; P30 control *vs.* P19 LT-NMDA: $p < 10^{-5}$, Kolmogorov-Smirnov test; P19 control = 71 cells from 7 mice, P30 control = 80 cells from 9 mice, P19 LT-NMDA = 129 cells from 11 mice) and retained OD plasticity (P19 LT-NMDA *vs.* P19 LT-NMDA MD: $p = 0.0003$, Dunn-corrected post hoc test; $p < 10^{-5}$, Kolmogorov-Smirnov test; P19 LT-NMDA = 129 cells from 11 mice, P19 LT-NMDA MD = 109 cells from 8 mice). Error bar indicates mean ± SEM. n.s., $p > 0.05$,*$p \leq 0.05$, **$p < 0.01$ and ***$p < 0.001$.

from contralateral to equal bias around P24, and the critical period for OD plasticity is from P14 to P30. During critical period, V1 feedback plays distinct roles in dLGN development.

The formation of equal OD bias in dLGN binocular neurons

Rodent V1 is dominated by the input from the contralateral eye. Previous study by McCurry et al.[35] showed that establishing OD bias in V1 required neuronal activity and visual experience, which is similar to the process of formation of equal OD bias in dLGN binocular neurons in our study. During this process from contralateral OD bias to equal bias, we found that though the response strength from both eyes reduced, the decline of the contralateral response was more robust than that of ipsilateral response. Previous studies demonstrated that there existed remodeling of the retinogeniculate projections after receiving visual

information.[31,32] Among this process, the retinogeniculate axon terminals projected onto dLGN neurons may eliminate, especially for the contralateral input, to form the equal OD bias of binocular dLGN neurons.

Interestingly, Sommeijer et al.[13] and Jaepel et al.[14] showed that dLGN displayed a contralateral OD bias as V1. We found, however, the response to the contralateral eye was equal to that of ipsilateral in mature dLGN binocular neurons, which is consistent with a recent study.[15] The differences between our results and the above two studies are as follows. First, we performed full-screen on-off stimulus, which may evoke more responsive neurons and stronger responses than orientation stimulus.[14] Second, we distinguished on and off response and compared ODI change only during on stimuli rather than merging them.[13] Although one dLGN neuron can receive inputs from both on and off retinal ganglion cells (RGC),[36] the ON and OFF pathways in retina are traditionally considered as two parallel pathways and asymmetric in some functions.[37,38] We think the strategy for synaptic summation of on and off responses may be different in dLGN neurons; thus, the mixture of on and off responses may interfere with the results.

Previous studies demonstrated that RGC axons innervated dLGN at perinatal ages, but after eye opening, synapses eliminate and 1–3 dominant retinal inputs drive each geniculate neuron.[31,32] These indicated that there existed remodeling of the retinogeniculate pathway after receiving visual experience. We tested the change of binocular retinal inputs by intraocular cholera toxin subunit β (CTB) injection and found that the fraction of dLGN covered by contralateral and ipsilateral retinal projections changed with age, but the ratio kept stable (data not shown). Thus, the remodeling of the retinogeniculate may participate in the formation of equal OD. To further elucidate the mechanisms contributing to this hypothesis, we could examine the change of molecular expression which related to the mapping of retinal projections, such as Ten-m3,[39–41] Ten-m2,[42,43] and ephrin[44] during development.

Critical period for OD plasticity in dLGN binocular neurons

Because it was previously accepted that the OD plasticity occurred exclusively in the cortex, there were few reports of OD plasticity in dLGN. Consistent with previous studies,[13,14] we observed significant OD shift caused by short-term MD. We further showed that the OD shift occurred exclusively within a specific time window (the critical period), similar to V1. The OD shift was mainly caused by a decrease of deprived-eye (contralateral eye) response, which agrees with the result of 2-day MD by Sommeijer et al.[13] Recent studies showed that 7-day MD in juvenile[13] and adult mice[14] induced an increase of non-deprived eye response. These imply that the mechanism of long-term MD may be similar to V1.[45–47] However, another study showed that 14-day MD from P19 disrupted the binocular integration and influenced the fraction of binocular neurons, not OD shift.[15] This raises the possibility that the different influences of MD on dLGN may be associated with its starting time and duration.

The mechanism of V1 feedback on thalamic development

In our study, we found three distinct roles of V1 feedback on thalamic development. Firstly, using muscimol to instantly inactivate V1, the spike characteristics were changed as reported before.[33,48] The increase of spike rates and reduction of response latency indicate that V1 may provide inhibition to dLGN neurons via TRN nuclei or local dLGN interneurons. Several studies showed that dLGN neurons had circular center-surround receptive fields that can be sharpened by V1 feedback.[26,48] Researchers further reported that the surround suppression is weakened by cooling or ablating the visual cortex,[49,50] and TRN may be involved with large-size stimulus.[51] Thus, we propose that the instant V1 inactivation led to the loss of suppression in TRN. When we performed full-screen on-off stimulus, the activity of dLGN neurons increased because of the decrease of surround suppression.

Secondly, with short-term V1 inactivation during development, we found that the equal OD bias was reversed to an immature contralateral bias, which was mainly caused by the increase of contralateral response. This suggests that during the normal dLGN development, V1 feedback contributes to the formation of equal OD bias in dLGN by reducing the contralateral response. Similar result was observed by Diao et al.,[22] in which they found that the contralateral retinogeniculate projections increased in Fezf2 conditional knockout (cKO) mice whose corticogenicultate connection was disrupted from embryonic stages. However, it is still unclear why the loss of V1 feedback led to the changes of contralateral responses specifically. One alternative explanation is that the process of refinement of dLGN circuits tends to prune the abundant inputs from the contralateral eye. Rompani et al.[9] showed that ipsilateral clusters receive input from few, selected types of ganglion cells and are therefore functionally specialized, while contralateral

clusters combine ganglion cell types more broadly in dLGN neurons. Thus, for a dLGN neuron of specific function, it could prefer to eliminate redundant inputs from the contralateral eye, during which process V1 may participate in.

At last, long-term V1 inactivation spanning the whole critical period retained both OD bias and plasticity in an immature state, which is similar to the developmental stage at the start time point of the V1 inactivation. This result suggests that long-term V1 inactivation pauses the following development of dLGN, which conforms to the important role of V1 feedback on dLGN development.[21,25,51] It seems that V1 not only affects the contralateral retinal inputs to form contralateral OD but also during the whole development after eye opening, V1 feedback guides the correct retinogeniculate connection through Hebbian plasticity. After disrupting the corticothalamic or thalamocortical projection, the retinal axons fail to target exact dLGN neurons, which affect the synapses' survival and axonal segregation pattern of dLGN.

Limitations of the study

There are also several limitations in our study. First, the area of V1 inactivation with pharmacological manipulation is not exactly the same across each mouse. The corticothalamic connection has retinotopic organization,[27,51] so the deviation of inactivated areas may result in variable results. Second, since we blocked the corticothalamic projections through inactivating V1 neurons, the efferent from dLGN to V1 was also affected with no postsynaptic firing. We cannot rule out the influence of dLGN development caused by incorrect efferent to V1. In addition, due to the limitation of electrophysiology, though we tried our best to cover the whole dLGN during recordings, our data could not include the border of thalamus to prevent sampling bias.

In this study, we found that there are two different processes, the formation of equal OD bias and OD plasticity, during a juvenile critical period development in dLGN binocular neurons, and V1 feedback plays distinct roles in the modulation of the two processes. Potential mechanisms regulating different stages of dLGN development require further investigation.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.105752.

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AUTHOR CONTRIBUTIONS

Conceptualization, N.L. and Y.G.; methodology, N.L. and Q.L.; formal analysis, N.L. and Q.L.; investigation, N.L., Y.Z., and Z.Y.; writing – original draft, N.L.; writing – review & editing, N.L., X.S., and Y.G.; supervision, X.S. and Y.G.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
urethane	Sigma-Aldrich, China	Cat# U2500-100G
chlorprothixene	Sigma-Aldrich, China	Cat# C1671-1G
DiI	Invitrogen, USA	Cat# D282
Muscimol hydrobromide	Sigma-Aldrich, China	Cat# G019-5MG
N-methyl-d-aspartate (NMDA)	Sigma-Aldrich, China	Cat# M3262-25MG
Experimental models: Organisms/strains		
C57BL/6 mouse	Shanghai Shrek Experimental Animal Co., Ltd	N/A
Software and algorithms		
Psychopy	Psychopy	https://www.psychopy.org/
Neuroexplorer	Plexon	https://plexon.com/products/neuroexplorer/
Python	Anaconda	https://www.anaconda.com/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr Yu Gu (guyu_@fudan.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

All data reported in this paper will be shared by the lead contact upon request.

This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The wild-type C57BL/6 mice were housed at 24°C, 12 h light and 12 h dark cycle, with food and water available *ad libitum*. Mice were weaned at P21 and co-housed with four or five littermates of the same sex. For all experiments, mice of both sexes were used. All protocols and procedures followed the guidelines of the Animal Care and Use Committee of Fudan University.

METHOD DETAILS

Monocular deprivation

Animals were anesthetized with isoflurane (5% for induction, 1–2% for maintenance in 1 L/min O_2). The margins of the upper and lower lids of the right eye (contralateral to the side on which recordings were performed) were trimmed and sutured together with three mattress sutures (6–0 silk, Ethicon).[52] Erythromycin ointment was applied twice a day after surgery to prevent inflammation of the wound. For all experiments, the duration of eye closure was 3 days or long-term monocular deprivation (LTMD, 7 days). Eye was further checked daily for proper closure. The suture was removed with fine scissors and examined under a stereomicroscope before electrophysiological recordings. Animals with eye opened prematurely, as well as animals with damaged eyes after eye re-opening, were excluded from the experiments.

In vivo electrophysiology

Mice were anesthetized with an intraperitoneal injection of urethane (20 mg/kg in 10% saline solution, Sigma-Aldrich) and then sedated with chlorprothixene intramuscularly (5 mg/kg in DMSO, Sigma-Aldrich).[53] The animal was placed on a stereotaxic frame (RWD Life Science, China) and maintained at 37°C by a feedback-controlled heating pad (Harvard Apparatus). Silicon oil was applied to both eyes to prevent them from drying. The scalp was shaved and skin removed to expose the skull.

A small craniotomy (~2 mm^2) was made on the left hemisphere skull directly above the dorsolateral geniculate nucleus (dLGN, 2.3–2.7 mm posterior from bregma and 2–2.3 mm lateral from midline) according to a stereotaxic mouse atlas.[13,36] A linear silicon electrode (16 channels, ASSY-1-16-1, Lotus Biochips, United States) or a 4-shanks multi-microelectrode (32 channels, NeuroNexus Technologies Inc, United States of America) was inserted into the dLGN at the depth about 2200–2600 μm. We recorded two or three sites of each mouse dLGN when used 16-channels electrode to covered the whole dLGN as far as possible. In a subset of recording, the primary visual cortex (V1) was also targeted (1.5–3.5 mm lateral from the midline and 0.3–0.8 mm anterior from the lambda suture) lowered about 0.6 mm. Signals were acquired using OmniPlex Neural Recording Data Acquisition System (Plexon Inc., Dallas, United States). Electrical signals were amplified and filtered from 0.3 to 6 kHz for spike, and 0.5–300 Hz for visual evoked potential (VEP). Sampling rate was 40 kHz. Throughout recordings, toe-pinch reflex was monitored and additional urethane was supplemented as needed. Two homemade tapes were put close to individual eyes and alternately open to achieve monocular visual stimulation during recordings. At the end of each recording, the animal was sacrificed for molecular and morphological experiments.

Reconstruction of recording sites

To verify the location of recording sites, we used histological reconstructions. Prior to insertion into dLGN, the electrode was coated with DiI, a fluorescent lipophilic tracer (Invitrogen). After the last recording, mice were perfused with phosphate buffered saline (PBS) solution and then the brain was post fixed in 4% paraformaldehyde overnight. Coronal tissue sections of 50 μm were cut with leica vibrotome (Leica VT 1200s, Germany) at RT and then stained in 1:1000 DAPI working solution for 10 minutes and washed for 2 times. At last, slides were mounted and fluorescent images were taken by fluorescent microscope (Olympus FluoView FVMPE-RS). The territory of dLGN can be visualized by DAPI and the DiI position of electrode sites were clearly demarcated.

Visual stimuli

Visual stimuli were generated with Psychopy v3.0 and presented on a monitor with gamma correction (Dell, 57 × 34 cm, 60 Hz refresh rate, ~100 cd/m^2 luminance), positioned 20 cm directly in front of the mouse. To measure the ocular dominance plasticity, we used on-off stimuli. The stimuli consisted of full screen white as on stimulus and grey as off stimulus (1.5s each) and repeated 12 times followed by a 3s gray blank condition (50% contrast) interval to estimate the spontaneous firing rate. For each eye, this stimuli block was repeated 5 times.

Pharmacological manipulation

For cortical instant inactivation, we made the second craniotomy ipsilateral to the hemisphere of recorded dLGN to expose the entire V1 (1.5–3.5 mm lateral from the midline and 0.3–0.8 mm anterior from the lambda suture). The muscimol (5mM in ACSF, Sigma-Aldrich), a $GABA_A$ receptor agonist to inhibit V1 activity, was topically applied to the surface of V1. After 10 minutes, the visual response abolished in all cortical layers without affecting subcortical structures (Figure 3A). Then the muscimol was washed out with ACSF to prevent drying and the effect of inactivation lasted at least 3 hours.

For short-term and long-term V1 inactivation, we lesioned V1 by injected N-methyl-d-aspartate (NMDA, an excitatory transmitter, can produce excitotoxic injury to kill cells)[22,34] into the left V1. For injections, Mice were placed in a plastic chamber, anesthetized with isoflurane in air (5% for induction, 1–2% for maintenance) and then fixed in a stereotactic frame (RWD Life Science, China). Body temperature was kept at 37°C by a feedback-controlled heating pad (Harvard Apparatus). Before the surgical procedure, the eyes were protected with Silicon oil. After a midline scalp incision, six small holes was made with a skull drill to cover the whole V1 located in the left hemisphere (1.5, 2.5, 3.5 mm latera from the midline and 0.3, 0.8 mm anterior from the lambda suture). A volume of 100 nL NMDA was injected at a depth of 0.3 mm

and 0.6 mm respectively by using NanoJect II (Drummond Scientific Company). The pipette was left in place for an additional 5 min to allow for diffusion. Finally, the wound was sutured. Following surgery, mice were placed on a heat pad to recover and monitored for postoperative health. After electrophysiological experiment, the lesion of V1 were observed through Nissl staining method to confirm the effect of NMDA.

QUANTIFICATION AND STATISTICAL ANALYSIS

Electrophysiological data analysis

Single units were detected and categorized using Offline Sorter (Plexon) by principal component analysis, whereby distinct clusters of spikes were identifiable with a high degree of similarity in a 3D feature space. And then Neuroexplorer (Plexon) was used for follow-up processing. All data were further statistically analyzed using a custom software written in python. For measuring firing rate, average number of spikes during the 1.5 s stimulation were calculated as response magnitude (R) with the spontaneous rate subtracted to obtain the response to a particular stimulation condition (on/off stimuli) across all trials. Visual responsive units were characterized as those that showed discernible peaks in peri-stimulus time histograms, by calculating the difference of distribution between stimulus and spontaneous spikes through the Kolmogorov-Smirnov test at the 1% significance level. In brief, we calculated peristimulus histograms for the above 1.5s stimulation (25 ms bin size), and compared the first 0.5s (20bins) distribution of stimulation with the first 0.5s spontaneous (gray blank condition interval, see STAR Methods) firing activity. The units of contralateral or ipsilateral response units were defined as firing during stimulation but significantly responding (pass the above test) to contralateral or ipsilateral eye only. Responsive latency was calculated as the time to half maximum response, based on mean-evoked firing rates.[54]

For the data of on-off stimuli, to quantify the degree of ocular dominance, the ocular dominance index (ODI) was calculated as $(R_{contra}-R_{ipsi})/(R_{contra}+R_{ipsi})$ only for binocular cells, where R_{contra} or R_{contra} represent the response magnitude caused by the on stimulus presented to the contralateral or ipsilateral eye. ODI ranges from 1 to −1, units were classified by classic seven-point scheme.[28] More specifically, Cells in group 1 which ODI ranged from −1 to −5/7 were driven only by the contralateral eye; for cells in group 2 which ODI ranged from −5/7 to −3/7 there was marked dominance of the contralateral eye, for group 3 which ODI ranged from −3/7 to −1/7 were slight dominance. For cells in group 4 which ODI ranged from −1/7 to 1/7 were there was bias between the two eyes. In group 5 which ODI ranged from 1/7 to 3/7 were the ipsilateral eye dominated slightly, in group 6 which ODI ranged from 3/7 to 5/7 were markedly; and in group 7 which ODI ranged from 5/7 to 1 were driven only by the ipsilateral eye. Correspondingly, the contralateral bias index (CBI) was then calculated according to the following formula: CBI = [(N1- N7) + (2/3) * (N2-N6) + (1/3) * (N3-N5) + N]/2N, where Nx was the numbers of cells with ocular dominance category equal to x and N was the total number of examined cells.

Statistics

Data are reported as median ± interquartile range (IQR), as mean ± s.e.m. or as mean ± s.d. as indicated in individual figures. For ODI comparison either in different ages or with different V1 inactivation treatments, to avoid the bias of induvial mice, we used nested t test to compare two groups and nested one way ANOVA for more groups comparison. Considering there is a large spread of ODI, we also compared the distribution of ODI by using Kolmogorov-Smirnov test. For evoked spikes and latency comparison, to avoid making assumptions about the distribution of the data, we used non-parametric tests for statistical comparison accordingly (Wilcoxon signed-rank test, Wilcoxon rank-sum test, Kruskal–Wallis test). All multiple comparisons were Dunn-corrected post hoc test. No randomization or blinding was performed during experiments or data analysis. Asterisks indicate significance values as follows: n.s., $p > 0.05$,*$p \leq 0.05$, **$p < 0.01$ and ***$p < 0.001$.