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NMDA receptors-dependent plasticity in the phototaxis preference behavior induced by visual deprivation in young and adult flies

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Adult mammals have experience-dependent plasticity in visual system, but it is unclear whether adult insects also have this plasticity after the critical period of visual development. Here, we have established a modified Y-maze apparatus for investigating experience-dependent plasticity in *Drosophila.* Using this setup we demonstrate that flies after the critical period have bidirectional modifications of the phototaxis preference behavior (PPB) induced by visual deprivation and experience: Visual deprivation decreases the preference of flies for visible light, while visual experience exerts the opposite effect. We also found an age-dependent PPB plasticity induced by visual deprivation. Molecular and cellular studies suggest that the *N*-methyl-D-aspartate receptors (NMDARs) mediate ocular dominance plasticity in visual cortex in mammals, but direct behavioral evidence is lacking. Here, we used the genetic approaches to demonstrate that NMDAR1, which is NMDARs subunit in *Drosophila,* can mediate PPB plasticity in young and adult flies. These findings provide direct behavioral evidence that NMDAR1 mediates PPB plasticity in *Drosophila.* Our results suggest that mammals and insects have analogous mechanisms for experience-dependent plasticity and its regulation by NMDAR signaling.

Keywords: Critical period, *Drosophila*, experience-dependent plasticity, NMDAR1, phototaxis preference behavior, visual deprivation

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Visual circuitry is a canonical model system for the study of neuronal plasticity and visual deprivation is a common manipulation for modulating cortical circuits. Previous studies have shown that the rats have experience-dependent plasticity in visual acuity into adulthood (Iny *et al*. 2006; Karmarkar & Dan 2006) and the goldfish also have rapid homeostatic plasticity in neuron activity of adult visual system (Riegle & Meyer 2007). However, experience-dependent plasticity in insects is thought to be limited to the critical period of visual development (Hirsch *et al*. 1990; Mimura, 1986). The first 4 days after eclosion is the critical period of visual development in *Drosophila* (Hirsch *et al*. 1990; Mimura 1986; Pyza 2002). Although *Drosophila* is a classical model organism because of its genetic and behavioral advantages, the mechanisms of experience-dependent plasticity in visual system remain unknown owing to the lacking of the effective setup.

In the present study, we established a modified Y-maze (Choe & Clandinin 2005; Harris *et al*. 1976; Quinn *et al*. 1974) to study experience-dependent plasticity in visual behavior induced by visual deprivation in *Drosophila* and found that flies after the critical period had the phototaxis preference behavior (PPB) plasticity. Our results suggest that flies have a greater potential for experience-dependent plasticity than previously appreciated.

The mechanisms of visual system plasticity in *Drosophila* lag behind the mammals. In the mammals, the mechanisms of experience-dependent plasticity at the cellular and synaptic levels have been studied very well because of the advantages of molecular biology and electrophysiology (Berardi *et al*. 2003; Hooks & Chen 2007; Philpot *et al*. 2001a). The *N*-methyl-D-aspartate receptors (NMDARs) are considered to be one of the key components in synaptic plasticity. The composition of NMDARs can be reversibly modified by visual experience and deprivation in visual cortex (Philpot *et al*. 2001a; Quinlan *et al*. 1999a,b). Adult ocular dominance plasticity requires NMDAR1 using the visually evoked potentials recording (Sawtell *et al*. 2003). However, there is no direct behavioral evidence to demonstrate that NMDAR1 mediates experience-dependent plasticity in visual system.

Because NMDAR1 in *Drosophila* has higher homolog with rat NMDAR1 (50%) compared with NMDAR2 (Xia *et al*. 2005) and NMDAR1 in the central brain mediates olfactory learning and memory in *Drosophila* (Xia *et al*. 2005) which are related to neuronal plasticity, we hypothesize that NMDAR1 might modulate PPB plasticity in *Drosophila*. To investigate this hypothesis, we took advantages of the GAL4/UAS system to modify the expression level of NMDAR1 (Chiang *et al*. 2002; Xia *et al*. 2005). We found that PPB plasticity was modulated by NMDAR1 homeostasis in young and adult flies. Our findings not only provide behavioral evidence of NMDAR1-dependent plasticity in visual system, but also suggest that

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NMDAR1 may play similar role in developmental and adult plasticity.

Materials and methods

Drosophila stocks

UAS-Nmdar1.Exel, *elav*-Gal4 and *tub*-GAL80^{ts} were obtained from Bloomington *Drosophila* Stock Center. P{EP} Nmdar1^{EP331} (EP331) was from the Szeged *Drosophila* Stock Centre. *hs*-Gal4 (P70) was kindly provided by Professor Li Liu.

Fly preparation

Wild-type flies were raised in standard medium at 25°C and 60% humidity in normal rearing (NR, 12-h light and 12-h dark cycle). Flies were collected within 1–2 days after eclosion, and then stochastically divided into two groups and reared in dark rearing (DR, constant darkness) or NR conditions respectively (Fig. 1b).

Heat-shock regimen

For heat-shock induction, flies were reared at 18°C and 60% humidity before collections. On the second day or the fourth day after collections flies were transferred to the empty vials which were pre-heated at 37°C in an incubator, for 1 h of heat-shock treatment at 37°C. Heat-shock treated flies were moved back to 25°C and 60% humidity for recovery. After 1–2 h recovery, flies were transferred to an incubator at 18°C and 60% humidity for experiments. Heat-shock treated flies were transferred to the test room for 1–2 h adaptation before experiments.

Behavior setup

Behavior setup consisted of three tubes, one tube in the left and two tubes in the right of the drawing. Small spots at the base of left tube indicate the position of flies before choice test. Two right tubes as choice tubes placed horizontally in the right of the drawing (Fig. 1a). Light source is light-emitting diode (LED). One was illuminated with blue light (460–465 nm) or green light (505–510 nm) and the other with red light (635–640 nm) or dark. We used light intensity meter to ensure light intensities of each choice tube at 500 lux. The two choice tubes were blacked out with opaque material.

Flies were tested in a dark room at 23–25°C and 60% humidity. At the beginning of each test, approximately 100 flies in left tube of the drawing (Fig. 1a) were restrained by a transparent baffle (the first door in Fig. 1a). At the same time, the second door (between two right tubes) was opened. After 30 seconds, the two colored lights at the base of right tubes were switched on and the first door was simultaneously opened. Flies moved phototactically into the two tubes immediately while lights were turned on. After 30 seconds, the first and second doors were closed at the same time. Flies in the choice tubes were collected separately and counted. This included the flies in the two pathways separated by the second door. The blue–red light choice setup was used in PPB experiments (Figs. 2–5 and Figure S2, Supporting Information).

Western blotting

The heads of adult flies were homogenized in lysis buffer (Xia *et al*. 2005) with protease inhibitor cocktail (11873580001, Roche, Basel, Switzerland). Homogenates were centrifuged at 19 064 ***g*** at 4°C for 50 min and the supernatants were collected. Total protein concentration was measured by Brandford method. Supernatants were separated by 6% SDS–PAGE and then transferred to PVDF membranes (0.45 mm, Millipore, Billerica, MA, USA). After 1 h blocking (5% skim milk), membranes were incubated with a mouse monoclonal antibody against rat NMDAR1 (mab363, Chemicon, Temecula, CA, USA; 1:1000) or rabbit anti-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA; sc-1616-R 1:2500) for about 12 h at 4°C. After washing, membranes were incubated with HRP-conjugated goat–anti-mouse IgG secondary antibody at a 1:5000 dilution (115-035-003, Jackson ImmunoResearch Laboratories, West Grove, PA, USA) or HRP-conjugated goat–anti-rabbit IgG secondary antibody at a 1:10000 dilution (111-035-003, Jackson). We used the chemiluminescent horseradish peroxidase (HRP) substrate (Millipore) to detect HRP and exposed membranes to Kodak MR films.

Electroretinogram (ERG) recording

Flies were inserted into pipette tips and their head was immobilized with UV gel. The recording microelectrode was filled with Ringer's solution and put onto the eye surface. The reference electrode was inserted into the fly abdomen. After dark adaptation the recorded potential was to baseline, and then broadband white light pulses which last 4 seconds were used to stimulate the eyes. Light intensity was stayed constant during the experiments. The signal was recorded with Axopatch 200B in current-clamp mode at 10 kHz. Data on each fly was averaged from about 10 trials.

Statistical analyses

The preference index (PI) was calculated from the following formula: $(A - B)/(A + B)$, where A represents the number of flies in the visible light arm and B is the number of flies in the other arm (Fig. 1a). Statistical significance levels in PI were determined by the independent samples t test ($^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). n.s. indicates no significant difference. Error bars represent the standard error of the mean (SEM). The statistical package SPSS 11.5 was used for statistical analyses.

Results

Experience-dependent plasticity in PPB induced by visual deprivation in Drosophila

To study experience-dependent plasticity induced by visual deprivation, we established a modified Y-maze apparatus. Flies in our setup (Fig. 1a) faced a choice between two tubes, one with visible light, the other in darkness or red light. We used this setup to test the PPB of flies. To assess the impact of visual deprivation on PPB, flies after collections were housed in DR (constant darkness) for 5 or 6 days after collections (Fig. 1b).

Because the absorption wavelength of various rhodopsins in *Drosophila* ommatidia is in the 300–600 nm region (Salcedo *et al*. 2003), flies have minimal ability to detect red light (>600 nm), but are sensitive to blue light and green light. Thus flies prefer visible light in the visible–dark light choice setup (Fig. 1c,d). *Wild-type Berlin* (*WTB*) and *Canton-S* (*CS*) flies reared in DR for 5 days showed a lower PI (detailed description in Materials and Methods) for green light than those reared in NR (12-h light and 12-h dark cycle) in the green–dark light choice setup (Fig. 1c). Flies had similar results in the blue–dark light choice setup (Fig. 1d).

To preclude the possibility that this preference to visible light may arise from light-associated heat perception, we tested the PI of flies in the visible–red light choice. As flies are insensitive to the red light, red light can be used to balance the potential heating effects without affecting sensitivity to visible light. The PI of flies in DR decreased markedly compared with that of flies in NR in the green–red light choice setup (Fig. 1e). Results were the same as in the blue–red light choice setup (Fig. 1f).

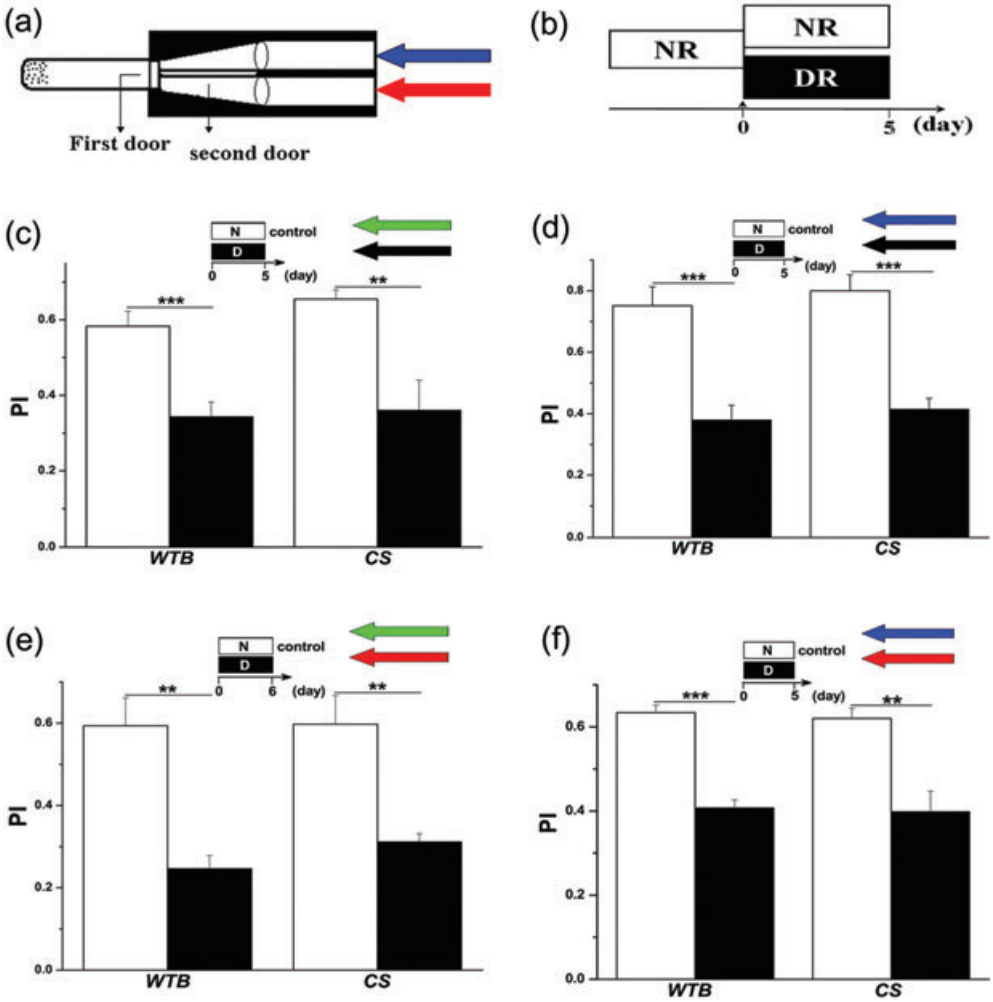

Figure 1: PPB of wild-type strains, *WTB* and *CS* in different light choice setup using the Y-maze. (a) Behavioral setup for PPB (see Materials and Methods for details). (b) Schematic of experimental protocol for visual deprivation. White bar indicates NR (N) and black bar indicates DR (D). Long horizontal black arrow indicates the development time of flies. Short vertical black arrow indicates the time point of collections (d0). (c–f) PPB of flies reared in DR and NR. $n = 5–7$ preference indexes per group. The schematic drawing in the top middle of every figure shows the experimental protocol. (c) PPB of wild-type flies in the green–dark light choice setup. (d) PPB of wild-type flies in the blue–dark light choice setup. (e) PPB of wild-type flies in the green–red light choice setup. (f) PPB of wild-type flies in the blue–red light choice setup. In all figures of the paper: DR is indicated by D and NR is indicated by N.

In order to determine whether the significant decrease in the PI of flies reared in DR was due to the changes of the locomotion activity, we monitored the activity of flies reared in DR and NR for 5 days. There were no significant differences in the locomotion activity of flies between DR and NR during the first day (Figure S1a) and the following 4 days (Figure S1b) after transferred to the activity monitor (Trikinetics Inc., Waltham, MA, USA). To study the effect of visual adaptation on the PI in our setup, we exposed NR flies to light or dark for half an hour before testing. In our setup light adaptation resulted in the decrease of PI and dark adaptation had opposite effect (Figure S2a). To further determine whether visual adaptation to light regime shift results in the significant decrease in the PI of DR flies, flies were exposed to light or dark for several hours before testing. The flies with pretreatment still showed significant differences in the PI of PPB between NR and DR (Figure S2b). PPB plasticity of flies induced by visual deprivation was independent of visual adaptation. In addition, previous studies indicate that visual deprivation for 4 days after eclosion fails to impair phototaxis in countercurrent selection (Benzer 1967; Hirsch *et al*. 1990; Mimura 1986). Thus, visual deprivation fails to significantly affect innate phototaxis in flies.

In summary, visual deprivation affected PPB of wild-type flies, which was not as a result of innate preference, heat effects, locomotion activity, visual adaptation or innate phototaxis. Thus the visible-red light choice setup using

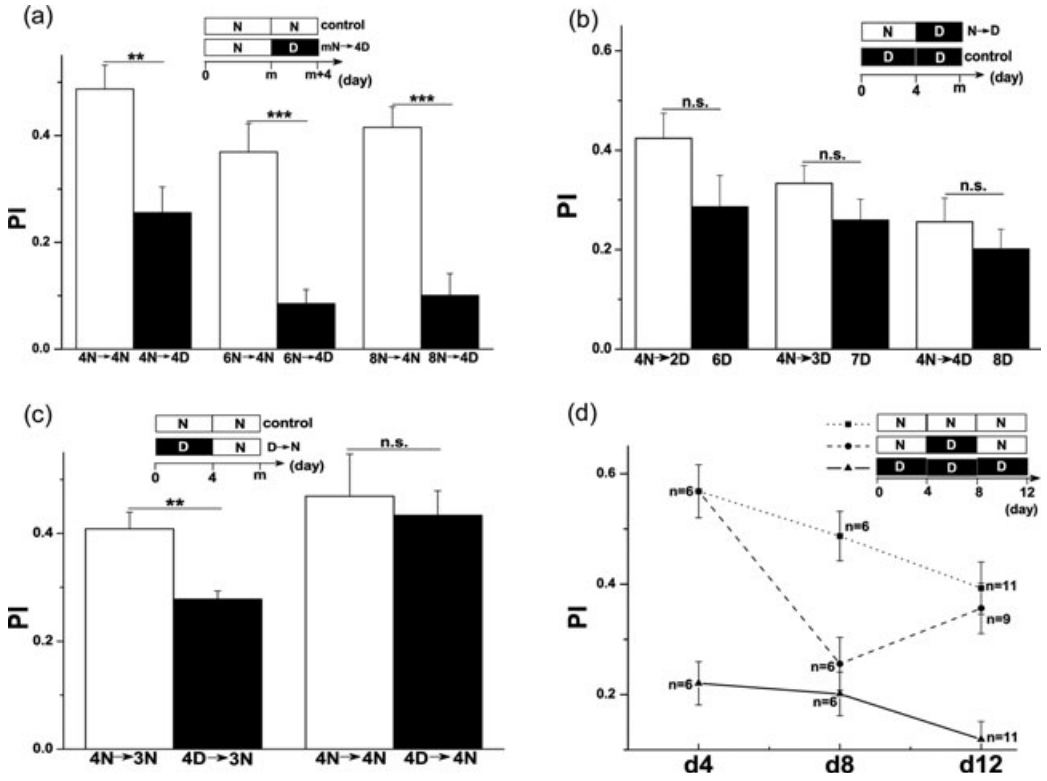

Figure 2: PPB of wild flies *CS* reared in DR and NR in the blue–red light choice setup. (a–d) $n = 5–11$ preference indexes per group. (a) PPB of flies after the critical period ($m = 4, 6, 8$). (b) The time course of PPB in flies induced by DR ($m = 2, 3, 4$). (c) The time course of PPB recovered by NR ($m = 3, 4$). (d) Bidirectional plasticity of PPB in flies induced by NR and DR. *n* indicates the number of preference indexes per group. The dot line denotes rearing of flies in NR (4N → 4N → 4N), the solid line denotes rearing of flies in DR (4D → 4D → 4D) and the dash line denotes rearing of flies in changing light environments (4N → 4D → 4N) in the top right corner schematic.

the Y-maze is an appropriate setup for studying behavioral plasticity in *Drosophila.*

Reversible plasticity of PPB in flies after a critical period

To further determine whether flies after the critical period maintain PPB plasticity, we reared flies in NR for more than 4 days and then transferred them to DR. Exposure to DR for 4 days before testing in these groups was sufficient to induce significant decreases in the PI compared with their controls (Fig. 2a). Above results suggest that adult flies after the critical period have PPB plasticity.

To further study whether visual deprivation after the critical period can decrease the PI to the level of the PI in flies always reared in DR since collections, we designed the following experiment. First we incubated flies in NR for 4 days after collections, and then transferred them to DR for different days. The 3–4 days of exposure to DR was enough to decrease the PI almost to the lowest level (Fig. 2b). Reciprocally, this effect was fully reversible when flies reared in DR for 4 days were returned to NR for several days (Fig. 2c), and this complete recovery required at least 4 days of exposure to NR ($P = 0.683$). Therefore, the PI in PPB of flies was modulated by visual deprivation and experience. Based on the above results, we designed bidirectional plasticity experiments (Fig. 2d). The PI of flies in 4D group markedly decreased compared with 4N group ($P < 0.001$). The PI of flies in 4N → 4D group showed a significant decrease compared with 4N → 4N group ($P < 0.01$) and dropped off quickly to almost that of 4D → 4D group ($P = 0.400$). After 4N → 4D treatment, flies were incubated in NR for 4 days, which was sufficient for the PI to recover to the level of 4N → 4N → 4N group flies ($P = 0.589$). The PI of 4N → 4D → 4N group flies showed a significant increase of PI compared with 4D → 4D → 4D group ($P < 0.001$) (Fig. 2d). Our results suggest that flies have reversible PPB plasticity after the critical period similar to that in mammals (Iny *et al*. 2006). In addition, we also observed that there was a gradual decrease in PI with age in flies that were reared in both DR and NR.

Age-dependent PPB plasticity induced by visual deprivation in Drosophila

Adult mice have age-dependent ocular dominance plasticity (Lehmann & Lowel 2008). In order to further study whether the flies have the similar phenomenon, we investigated the

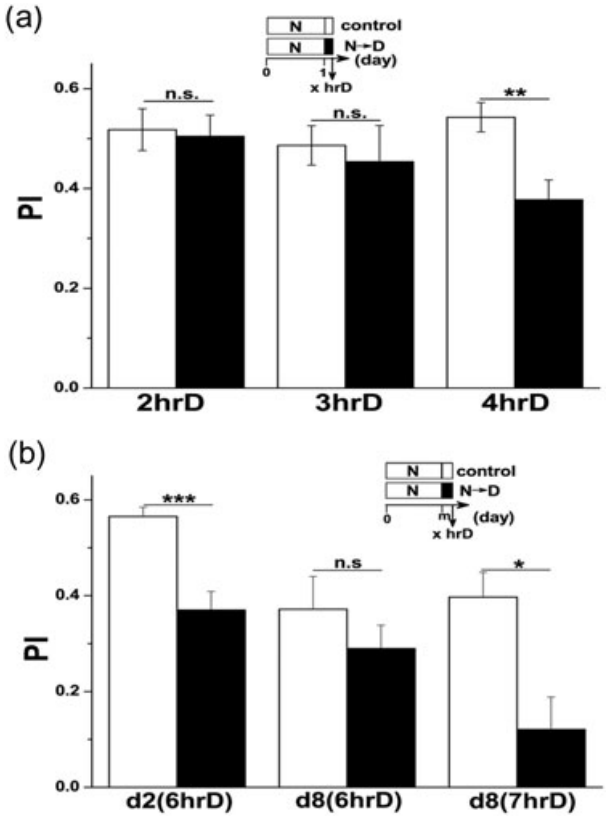

Figure 3: The time course of PI in wild flies *CS* induced by visual deprivation in the blue–red light choice setup. $n = 5–7$ preference indexes per group. Black arrow indicates the time point for testing. (a) PPB of 1-day-old flies with different hours in DR before testing ($x = 2, 3, 4$). (b) PPB of 2-day-old or 8-day-old flies with different hours in DR before testing ($m = 2$ or 8; $x = 6$ or 7).

time course of the PI in flies induced by DR. We exposed 1-day-old flies to DR for different lengths of time and found that 1-day-old flies required at least 4 h of dark exposure to achieve significant decrease in PI (Fig. 3a). DR for 6 h was enough to produce significant decrease in PI of 2-day-old flies, but failed to decrease the PI of 8-day-old flies compared with corresponding control. However, 7 h DR had significant effect on 8-day-old flies (Fig. 3b). The older the flies were, the more time of dark exposure was required to achieve significant difference in the PI of PPB. Therefore, our results revealed that there was an age-dependent PPB plasticity induced by visual deprivation.

PPB plasticity is modulated by up-regulation of NMDAR1

We found that PPB plasticity was induced by DR in above experiments. Because previous reports have shown that NMDAR1 level is up-regulated in visual cortex of DR mice (Tropea *et al*. 2006), we hypothesize that overexpressing NMDAR1 level may modulate PPB plasticity. To investigate this hypothesis, we used the pan-neuronal driver (*elav*-Gal4) to overexpress NMDAR1. The PI of overexpressing NMDAR1 flies in DR for different days had no significant difference compared with their corresponding NR groups (Fig. 4a). In order to exclude the developmental effect before eclosion, we crossed the *hs*-Gal4 with UAS-Nmdar1. Exel and activated NMDAR1 conditionally in their offspring by heat shock to exert precise temporal control. Because PPB plasticity may share different mechanisms before and after the critical period, we decided to activate the expression of NMDAR1 in different time (Fig. 4b,d). First we treated UAS-Nmdar1.Exel/+;*hs*-Gal4/+ (*hs*-Gal4/UAS-Nmdar1.Exel) flies with heat shock on the second after collections (Fig. 4b). After that, we transferred these flies separately to DR or NR. Heat-shocked flies showed no marked difference in PI between DR and NR ($P = 0.228$). Flies without heat-shock treatment were used as negative controls. Because overexpressing NMDAR1 flies in NR showed lower PI, it remained to detect whether these flies had normal photosensitivity. We found that overexpressing NMDAR1 flies showed normal response by ERG recording (Figure S3). Above results suggest that PPB plasticity is modulated by up-regulation of NMDAR1.

Furthermore, we used UAS-Nmdar1.Exel in combination with *tub*-Gal80^{ts}. We reared UAS-Nmdar1.Exel/+;*tub*-Gal80^{ts}/+;*elav*-Gal4/+ (UAS-Nmdar1.Exel;Gal80^{ts}/*elav*-Gal4) flies at 18°C to ensure the suppression of NMDAR1 expression before collections (Fig. 4c). And then these flies were transferred to incubator at 29°C on the second day after collections. After 1 day, flies were incubated in DR and NR for 3 days in the same incubator at 29°C. There were no significant differences in the PI of these flies between NR and DR ($P = 0.463$). Therefore, results from UAS-Nmdar1.Exel;Gal80^{ts}/*elav*-Gal4 flies further confirmed the consequence in *hs*-Gal4/UAS-Nmdar1.Exel flies during the critical period.

Because up-regulation of NMDAR1 modulated PPB plasticity during the critical period, it remained unknown whether NMDAR1 modulated PPB plasticity in flies after the critical period. We collected and reared *hs*-Gal4/UAS-Nmdar1.Exel flies in NR for 4 days at 18°C, and then treated them with heat shock (Fig. 4d). After that, flies were reared in DR or NR. No significant difference in PI of flies was shown between DR and NR ($P = 0.161$), which indicates that the overexpression of NMDAR1 also modulates adult PPB plasticity. To test the efficiency of UAS-Nmdar1.Exel, western analysis revealed that the NMDAR1 protein was enhanced in UAS-Nmdar1.Exel;Gal80^{ts}/*elav*-Gal4 (Fig. 4e) and *hs*-Gal4/UAS-Nmdar1.Exel (Fig. 4f) flies. Taken together, our results suggest that PPB plasticity is modulated by up-regulation of NMDAR1 in young and adult flies.

PPB plasticity is blocked by down-regulation of NMDAR1

After investigating up-regulation of NMDAR1, we examined the function of endogenous NMDAR1 by decreasing its expression level and checking its influence on PPB plasticity in *Drosophila.* To exclude the possibility that

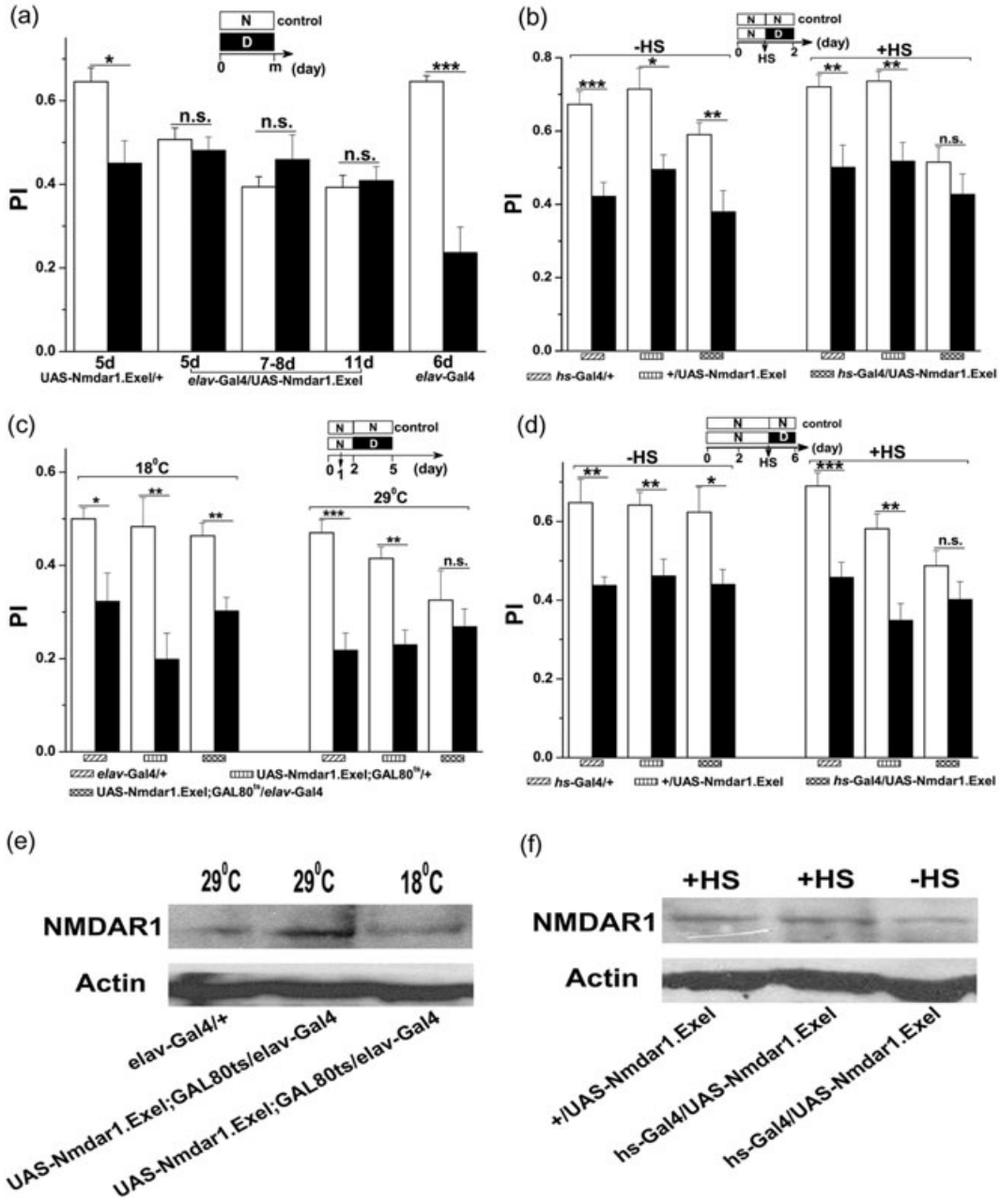

Figure 4: PPB of overexpressing NMDAR1 flies reared in DR and NR in the blue–red light choice setup. $n = 5–12$ preference indexes per group. (a) PPB of UAS-Nmdar1.Exel/+;*elav*-Gal4/+ (*elav*-Gal4/UAS-Nmdar1.Exel) flies reared in DR and NR. *m* indicates the time point of testing. For control, *elav*-Gal4 or UAS-Nmdar1.Exel was crossed to wild-type *CS* flies (+/UAS-Nmdar1.Exel and *elav*-Gal4/+). (b) PPB of UAS-Nmdar1.Exel/+;*hs*-Gal4/+ (*hs*-Gal4/UAS-Nmdar1.Exel) flies during the critical period with heat shock. For control, *hs*-Gal4 or UAS-Nmdar1.Exel was crossed to wild-type *CS* flies (*hs*-Gal4/+ and +/UAS-Nmdar1.Exel). The vertical arrow denotes the time point of heat shock (+HS) on the second day after collections. Flies not given heat shock are indicated by −HS. (c) PPB of UAS-Nmdar1.Exel/+;*tub*-Gal80^{ts}/+;*elav*-Gal4/+ (UAS-Nmdar1.Exel;Gal80^{ts}/*elav*-Gal4) flies during the critical period under the temperature control. For control, *elav*-Gal4 or UAS-Nmdar1.Exel;Gal80^{ts} in this figure was crossed to wild-type *CS* flies (UAS-Nmdar1.Exel;Gal80^{ts}/+ and +/*elav*-Gal4). The vertical arrow denotes the time point of transferring to 29°C in right groups. Flies in left groups always reared in 18°C. (d) PPB of *hs*-Gal4/UAS-Nmdar1.Exel flies after the critical period with heat shock. The vertical arrow denotes heat shock (+HS) on the fourth day after collections. The genotypes of heterozygous control flies were same with control flies in Fig. 4b. (e–f) Western blotting against NMDAR1 in UAS-Nmdar1.Exel;Gal80^{ts}/*elav*-Gal4 (e), *hs*-Gal4/UAS-Nmdar1.Exel (f) flies and their controls. Actin loading control is included.

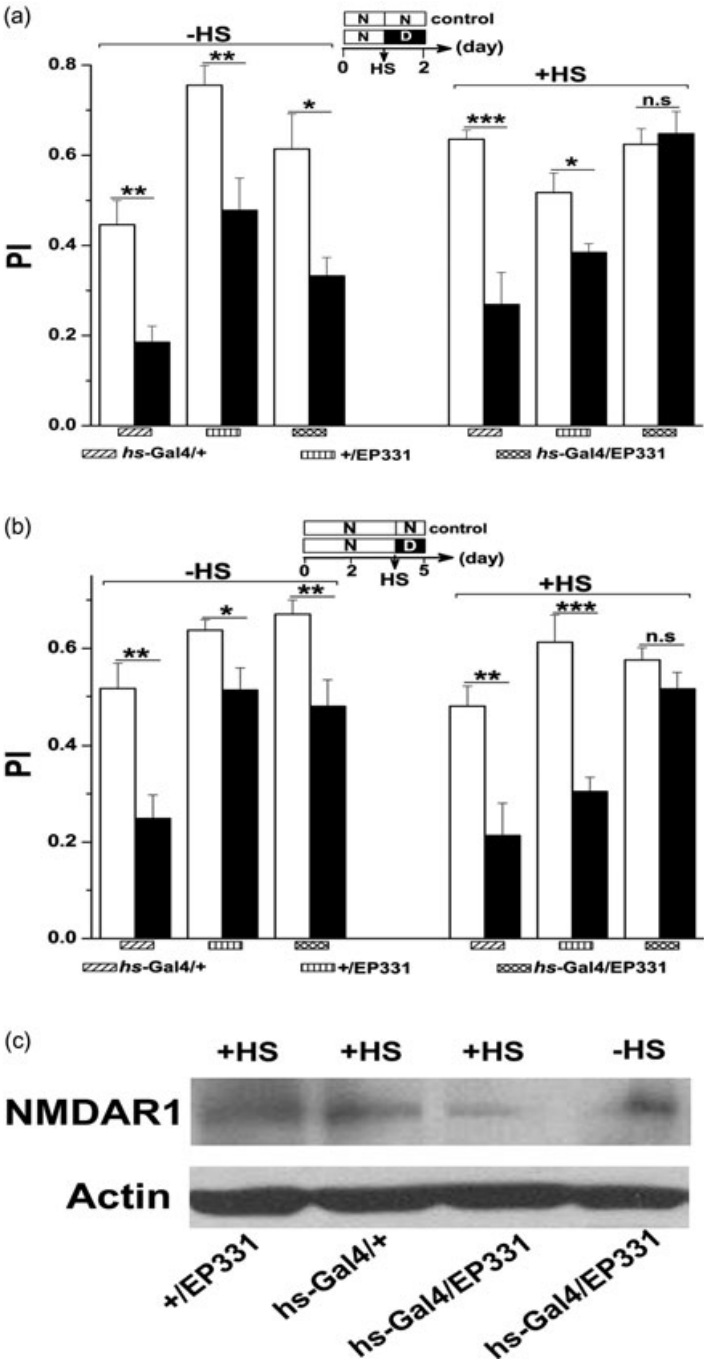

Figure 5: PPB of flies with down-regulation of NMDAR1 reared in DR and NR in the blue–red light choice setup. $n = 5–12$ preference indexes per group. (a) PPB of *hs*-Gal4/+;EP331/+ (*hs*-Gal4/EP331) flies during the critical period with heat shock. The vertical arrow in the schematic drawing in the top middle denotes the time point of heat shock (+HS) at the next day after collections. (b) PPB of *hs*-Gal4/EP331 flies after the critical period with heat shock. The vertical arrow in the schematic drawing in the top middle denotes the time point of heat shock (+HS) at the fourth day after collections. (a–b) For controls, EP331 was crossed to wild-type *CS* flies (EP331/+) and *hs*-Gal4 was crossed to Cantonized w^{1118} flies (*hs*-Gal4/+). (c) Western blotting against NMDAR1 in *hs*-Gal4/EP331 flies and their controls. Actin loading control is included.

reduced NMDAR1 affected plasticity by impairing development, we crossed the *hs*-Gal4 line with P{EP} Nmdar1^{EP331} (EP331) which was inserted by EP element at downstream of the NMDAR1 transcription unit (Xia *et al*. 2005). *hs*-Gal4/+;EP331/+ (*hs*-Gal4/EP331) flies with heat shock produced an antisense transcript of NMDAR1. First we disrupted the NMDAR1 of *hs*-Gal4/EP331 flies on the second day after collections with heat shock (Fig. 5a). These flies showed no significant decrease in PI in DR compared with control flies during the critical period ($P = 0.704$). This result suggests that PPB plasticity of flies is inhibited by down-regulation of NMDAR1 during the critical period.

And then, we reared *hs*-Gal4/dsNR1 flies in NR for 4 days at 18°C after collections (Fig. 5b). After that these flies were treated with heat shock and transferred to DR and NR. We found that these flies had no significant decrease between NR and DR ($P = 0.170$). This result suggests that NMDAR1 is indispensable to PPB plasticity in flies after the critical

period. The efficiency of *hs*-Gal4/EP331 flies was measured by western blotting. The expression level of NMDAR1 was reduced in *hs*-Gal4/EP331 flies with heat shock (Fig. 5c). Our results demonstrate that PPB plasticity is inhibited by down-regulation of NMDAR1 before and after the critical period.

Discussion

In summary, we have established a modified Y-maze apparatus to study experience-dependent plasticity in visual behavior in *Drosophila* and have showed that flies after the critical period of visual development have reversible PPB plasticity. In particular, we provide direct behavioral evidences that the homeostatic levels of NMDAR1 play an important role in PPB plasticity both in young and adult flies. The visible–red light choice setup described here provides a simple and appropriate paradigm for uncovering the mechanisms of experience-dependent plasticity.

Experience-dependent plasticity of visual system in Drosophila

Here, our finding that adult flies have reversible PPB plasticity is consistent with the studies on experience-dependent plasticity in mammals (Iny *et al*. 2006; Sawtell *et al*. 2003). However, previous studies show that behavioral plasticity occurs only within the critical period of visual development in *Drosophila* (Hirsch *et al*. 1990; Mimura 1986). Visual deprivation fails to affect the simple phototaxis of flies (Hirsch *et al*. 1990), but affect the phototaxis preference of flies in our choice setup. Compared with previous behavior assays such as countercurrent selection, flies in the visible–red light choice setup have to make a choice in the face of visible and red light, which is a more complex process than the simple phototactic attraction to light in countercurrent selection. This may be the reason why we have detected a significant change in PPB of flies after the critical period reared in DR. Our results suggest that the visible–red light choice setup is more effective than previous assays in detecting behavioral plasticity.

Besides behavioral studies, there are also structural studies on experience-dependent plasticity in *Drosophila.* Dark rearing can change size and fiber number of the optic lobes in *Drosophila* (Barth *et al*. 1997). It is generally considered that fly visual circuits are hardwired after visual development (Ting & Lee 2007) and visual deprivation after eclosion has no effect on the dendrites development in VS1 neurons in *Drosophila* (Scott *et al*. 2003). But our results indicate that visual deprivation after the critical period still affects PPB of adult flies. We propose that visual deprivation has a significant influence on fine visual perception such as color vision which results in the decrease of PI in PPB. The PPB plasticity observed by examining the effects of visual deprivation may result from alterations in precise synapse level rather than in major structural alterations of neuronal circuits (Destexhe & Marder 2004). Our findings suggest that insects may be similar to the mammals in adult experience-dependent plasticity induced by visual deprivation.

NMDAR1 homeostasis and experience-dependent plasticity in PPB

Although flies with up- or down-regulation of NMDAR1 had no significant difference in the PI of PPB between NR and DR, their mechanisms may be different. We observed that the PI of flies with overexpressing NMDAR1 in NR was similar to that of flies with expressing normal-levels of NMDAR1 in DR (Fig. 4). Because after DR the expression level of NMDAR1 is up-regulated in visual cortex in mice (Tropea *et al*. 2006), we suppose that overexpressing NMDAR1 flies in NR may simulate the situation of PPB in expressing normal-levels NMDAR1 flies induced by visual deprivation. Visual deprivation attenuates the increase of NR2A/NR2B (NMDAR2 subunits) ratio in visual cortex in rats (Philpot *et al*. 2001a; Quinlan *et al*. 1999a,b), but it is remained to be shown that whether overexpression of NMDAR1 can modulate NMDAR2 subunits ratio. In addition, we also demonstrate that down-regulation of NMDAR1 can affect PPB plasticity in young and adult flies. This result is in accordance with previous studies showing that ocular dominance plasticity in adult visual cortex of mice is blockaded in NMDAR1 knockout mice (Sawtell *et al*. 2003). Down-regulation of NMDAR1 blockades the NMDARs currents because of NMDAR1 and NMDAR2 subunits together to form functional NMDA channels in *Drosophila* (Xia *et al*. 2005). Therefore, we propose that down-regulation of NMDAR1 blockades PPB plasticity. Up- or down-regulation of NMDAR1 had similar behavioral effects both in young and adult flies. Our findings suggest that NMDAR1 homeostasis is indispensable for maintaining PPB plasticity in young and adult flies.

However, our above suggestions are based on the results of regulating NMDAR1 in the whole neurons. NMDARs in different regions of brain may have various functions for PPB plasticity. For example, overexpression of NR2B (NMDAR2 subunit) enhances the ability in learning and memory in mice (Tang *et al*. 1999). But overexpression of NR2B fails to alter the synaptic plasticity of the visual cortex in mice (Philpot *et al*. 2001b). Thereby it remains to be shown which regions are involved in the modulation of PPB plasticity, and whether or not NMDAR2, which is required for long term memory (Wu *et al*. 2007), has a similar function in experience-dependent plasticity in *Drosophila*.

Taken together, our results indicate that homeostatic levels of NMDAR1 are essential for modulation of experience-dependent plasticity and imply an inverted U-rule for the role of NMDAR1 in mediating PPB plasticity. This inverted U-rule is consistent with the role of dopamine levels in the modulation of complex behaviors in *Drosophila* (Andretic *et al*. 2005; Liu *et al*. 2008, 2009). By inverted U-rule, we mean that low or high levels of protein molecular will affect PPB plasticity. Actually, we found that PPB plasticity requires optimal levels of NMDAR1. High or low levels of NMDAR1 will block PPB plasticity. The effect of the expression levels of NMDAR1 on PPB plasticity can be qualitatively described

as an inverted U-curve of the NMDAR1 levels versus PPB plasticity.

In conclusion, we have established *Drosophila* as a model for investigating the mechanisms of experience-dependent plasticity in the visible–red light choice setup and have shown that PPB plasticity is exhibited well even after the critical period. Our findings suggest that fly neural circuits may be plastic to the environment in a similar way to the mammals, and NMDAR1 may play similar role in developmental and adult plasticity in visual behavior.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1: Locomotion activity of wild-type flies, *WTB* and *CS* reared in DR and NR. (a–b) Vertical coordinates indicate the average counts per minute (Avg_Counts/min). (a) Locomotion activity of flies at d5–6. Left bars: $n = 13, 16$ flies per group. Right bars: $n = 32, 31$ flies per group. (b) Locomotion activity of flies at d6–10. Left bars: $n = 13, 16$ per group. Right bars: $n = 32, 31$ per group.

Figure S2: PPB of wild flies *CS* after light and dark exposure in the blue–red light choice setup. $n = 5–6$ preference indexes per group. (a) PPB of flies reared in NR after light (L) or dark (D) exposure for half an hour. (b) PPB of flies reared in DR and NR after several hours of pretreatment in light (L) or dark (D) environments. Gray regions in the top left corner drawing indicate the pretreatment course before testing. x h indicates the time of pretreatment.

Figure S3: The photosensitivity of overexpressing NMDAR1 flies by ERG recording. (a) Sample trace from a fly in +/UAS-Nmdar1.Exel. dV indicates the maximum voltage after switching light on. dt indicates the time to reach the maximum voltage. (b–c) $n = 8$ preference indexes per group. For controls, UAS-Nmdar1.Exel was crossed to wild-type *CS* flies (+/UAS-Nmdar1.Exel). (b) dV of *hs*-Gal4/UAS-Nmdar1.Exel flies and their controls (+/UAS-Nmdar1.Exel) after heat shock ($P = 0.476$). (c) dt of *hs*-Gal4/UAS-Nmdar1.Exel and +/UAS-Nmdar1.Exel flies after heat shock ($P = 0.0218$).

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