Experience-dependent Plasticity of the Optomotor Response in *Drosophila melanogaster*

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Key Words

Optomotor response · *Drosophila melanogaster* · Visual system · Dark-fly · Plasticity

Abstract

Experience in early life can affect the development of the nervous system. There is now evidence that experience-dependent plasticity exists in adult insects. To uncover the molecular basis of plasticity, an invertebrate model, such as *Drosophila melanogaster*, is a powerful tool, as many established genetic and molecular methods can be applied. To establish a model system in which behavioral plasticity can be examined, we investigated the optomotor response, a behavior common to most sight-reliant animals, in *Drosophila* and found that the response could be modified by the level of light during rearing. The angle turned by the head in response to a moving stimulus was used to quantify the response. Deprivation of light increased the response to low-contrast stimuli in wild-type *Drosophila* at 4 days after eclosion and this plastic change did not appear in *rutabaga*, a known mutant defective in short-term memory. In addition, the change was transient and was markedly decreased at 6 days after eclosion. Further, we found that Dark-flies, which have been kept in constant darkness for more than 50 years,

showed a higher response to low-contrast stimuli even at 6 days after eclosion compared to wild type and this characteristic was not lost in Dark-flies placed in a normal light environment for 2 generations, suggesting that this high response has a hereditary nature. Thus, our model system can be used to examine how the environment affects behaviors.

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Introduction

The environment in which animals are reared often affects their responses and behavior. For example, cats reared in restricted visual environments show specific morphological alterations in the visual cortex [1, 2]. Such changes in the nervous system, which are called experience- or activity-dependent plasticity and require neural activity, have been extensively studied in many organisms, including invertebrates and vertebrates. Experience is also important for appropriate neural development in adult insects. After eclosion, deprivation of light impairs the development of visual pattern discrimination in the fly *Boettcherisca peregrina* without impairing phototaxis [3]. *Drosophila melanogaster* reared under enriched conditions have more Kenyon cell fibers in the peduncle of

the mushroom body than their deprived siblings [4, 5]. Experience-dependent developmental plasticity has also been reported in the optic lobe in *Drosophila* [6]. These studies have established the importance of environmental inputs and neural activity in the development and maturation of the neural system. Further, in mammals, it was found that experience during a certain period of development affects neural function throughout later life [7, 8]; this period is called the critical period and has now been found to occur in many organisms, including *Drosophila* [6, 9]. Light deprivation-induced changes in the volume of the optic lobe in *Drosophila* are also irreversible and have a critical period [6], while those induced in synaptic contacts in the lamina in the fly *Musca domestica* are reversible [10]. The mechanisms by which plastic changes induced during a certain period become fixed in later life have not been fully clarified.

Concerning the fixation of plastic changes, the examination of effects of long-term change in environment beyond generations is interesting. Mori [11] maintained the strains of *D. melanogaster* under constant darkness (DD) for many generations and showed several interesting changes in characteristics, such as in phototaxis (the phototactic response to light of different wavelengths), the fine structure of the compound eye, the type of daily eclosion rhythm, and the length of head bristles [11]. In particular, in flies reared in DD for 81 generations and brought back to a normal light environment, the altered character of moving actively towards a light source was not lost, even after 117 generations on a normal light cycle, suggesting that this change of behavior has a hereditary nature [12]. Additionally, genomic alterations have been recently reported in these flies that have been maintained in DD for 57 years [13].

In this study, we examined the acute effects of light deprivation on a behavior based on vision, the optomotor response, in adult *D. melanogaster*. We use *Canton-S* (CS) wild-type *D. melanogaster* and *rutabaga* (*rut*) mutants reared under conditions of either 12 h light-12 h dark (LD) or DD after eclosion; *rut* encodes calcium/calmodulin-responsive adenylate cyclase and its mutations have been shown to cause a deficiency in associative learning behavior, synaptic stability and plasticity, suggesting the importance of cAMP pathways in neural plasticity and function [14, 15]. Further, we also examined the response of Dark-fly Tokyo (hereafter referred to simply as Dark-fly, DF), a strain of *D. melanogaster* that has been cultivated in DD for more than 50 years, corresponding to at least 1,300 generations [16, 17]. The optomotor response is a behavior common to most sight-reliant animals

which allows them to move their eyes to keep the visual world stationary on the retina when they are exposed to moving objects. The neural mechanisms underlying this response have been extensively studied in the house fly and in *Drosophila* [18, 19]. The results presented here show the existence of cAMP signaling-dependent plasticity in *Drosophila* vision-based behavior, which may help it to adapt to long-term environmental changes.

Materials and Methods

Fly Stocks

D. melanogaster flies were reared at room temperature (25°C). CS was used as the wild-type fly. The *rut* mutant was provided by Dr. C.-F. Wu. DF was kindly provided by Dr. Michio Imafuku (Department of Zoology, Kyoto University). DF has been maintained in complete darkness at Kyoto University since the 1950s [16]; the DF stock used in this study was originally established from the Tokyo strain of *D. melanogaster*. Unfortunately, the original strain and the control strain reared on a light-dark cycle have been lost, so we compared DF with CS. DF was reared in DD and observed in a dark room under red light (approx. 700 nm) during rearing. Female flies were used for all experiments.

Optomotor Response

We measured the head-turning angle as an indicator of the optomotor response (see fig. 1). Previously, the fly's behavior on detecting motion has been measured by measuring torque [18, 20, 21], number of wing beats [22], the direction of walking [23], or the head-turning angle [21, 24, 25]. The advantage of measuring the head-turning angle is that it is applicable to mutant flies, which sometimes have problems in flying and walking. A fly was anesthetized on ice and a fine wire attached to its thorax with glue. Then, to minimize the body movement, the legs were stuck together with nail polish (see fig. 1a). This did not disturb the head movement. The fly was then tethered at the center of a circular arena made from paper (diameter 12.8 cm) on which was printed a striped pattern with a defined contrast and pattern wavelength (λ), measured in degrees based on the angle between the two lines from the center of the arena to the edges of a stripe. The arena was manually rotated around the animal on a turntable at a constant speed in alternate clockwise and counterclockwise directions, for 10 s each (see fig. 1c). We also used a 12-panel LED insect arena system (Metrix Technology Corp., New York, N.Y., USA) to compare the results obtained with those seen using the printed pattern (see Results). Optomotor responses (head movements) during visual stimulation were recorded on a video camera placed above the arena. After taking the video, we analyzed the optomotor response by measuring the head-turning angle. As shown in figure 1b, we determined the center line of the body with the fly facing forward and measured the angles between this center line and the inner edge of the compound eye with no stimulus and during visual stimulation using tracing software (PTV, Digimo, Japan), with the difference, θ , representing the head-turning angle. We used the data for the second half (5 s) of each 10 s period of visual stimulation and calculated the mean angle for one stimulus. Since each fly might have a preferred direction in which to turn, we cal-

Fig. 1. Measurement of the head-turning angle as an indicator of optomotor response. **a** Schematic drawing of how a fly was fixed with a wire and its legs were tied with nail polish. **b** Schematic drawing of a fly turning its head. The center line is defined in each fly with the head pointing forward, and the angle between this line and a line from the base of the head to the inner edge of the eye is measured by tracing. During each stimulus, the new angle between these lines is measured and the initial angle subtracted to give θ , the turning angle. **c** Example of a plot of the turning angle (θ ; top panel) during clockwise (CW) and counter-clockwise (CCW) stimulation (bottom panel).

culated the average of the responses to both clockwise and counterclockwise rotations, normalized it by the maximum head-turning angle of the fly, and averaged the normalized values for 3 trials per 1 fly. A larger head-turning angle means a larger response. Optomotor responses were measured during the daytime (from 11 a.m. to 6 p.m.) under light filtered with red cellophane paper in the dark room. Flies measured under the same background illumination were compared. Statistical analysis was performed by one- and two-way ANOVA and Student's *t* test. Optomotor responses are illustrated in figure 2.

Visual Stimuli

Square-wave patterns of the desired spatial wavelength (λ) at different levels of contrast (ratio of black to white) were designed using Adobe Photoshop and were printed as black and white stripes on white paper using Canon (Tokyo, Japan) printers, a Lasershoot LBP2810 printer for the experiments shown in figure 3a, b, and an iR-ADV C5035 printer for the experiments in figures 3c, 4 and 5. The same printed patterns were used for all animals that were compared, since results obtained using the same pattern printed on different printers or on the same printer at different times cannot be compared. The size of stripes was indicated as an angle of λ . We defined contrast as the ratio of intensity of the black stripe to that of the white stripe, 0% contrast being a black paper with no stripe. We also used a green LED arena (12 panels, diameter 10 cm) for the visual stimulus to compare the responses to those obtained using the printed patterns. In the LED arena, 'contrast' means the difference in intensity of the green light (568 nm).

Temporal frequency was defined as the rotation speed divided by the spatial wavelength of the stimulus, measured in degrees as described above. A temporal frequency of 4 Hz and pattern wavelength of 36° were used in all studies except those in figure 2.

Results

We first verified our visual stimulation and response quantification methods by examining whether the response had properties corresponding to those of elementary motion detectors of the correlation type, called Reichardt detectors [26, 27]. In this detector model, directionally selective responses are computed from the changing retinal brightness distribution by correlating, at each image location, the brightness values as derived from neighboring photoreceptor signals after asymmetric temporal filtering. This operation is performed twice in a mirror-symmetrical fashion before the outputs of both multipliers are subtracted from each other [26, 28]. This leads to a fully directional output signal. Therefore, these detectors respond maximally to a certain number of images crossing a single photoreceptor and not to a certain

Fig. 2. Optomotor response to a striped pattern stimulus printed on paper or generated by LED lightning in the wild-type CS fly at 4 days after eclosion reared under LD conditions. **a, b** Dependence of the response on the temporal frequency of stimulation using the printed pattern (**a**) or the LED pattern (**b**). The spatial wavelengths of the stimuli, measured as an angle as described in Methods, were ∇ 180° (n = 5), $\blacktriangledown$ 72° (n = 5), $\circ$ 40° (n = 9), and $\bullet$ 21° (n = 7) in **a** and $\blacktriangledown$ 120° (n = 5), $\circ$ 60° (n = 5), and $\bullet$ 22° (n = 5) in **b**. **c** Dependence of the optomotor response on the contrast of the stimuli printed on paper (n = 16) or LED lightning (n = 12). *yw* flies do not show a response (n = 10). Data represent the mean $\pm$ SEM.

speed at which the image crosses the photoreceptor, resulting in a constant temporal frequency optimum. In other words, the model using Reichardt detectors predicts a counterintuitive dependence of the motion detection process on pattern properties, such as its spatial wavelength and contrast. These characters have been demonstrated in the house fly *M. domestica* [29, 30] and in *D. melanogaster* [20], suggesting Reichardt motion detectors underlie motion vision in these animals. In this study, we measured the head-turning angle in response to a moving stripe stimulus and quantified the optomotor response as described in Methods and shown in figure 1. Wild-type CS flies at 4 days after eclosion reared under LD conditions were placed either at the center of a circu-

lar arena made of paper and subjected to stimulation by a striped pattern printed on the paper or at the center of an LED arena with stimulation provided by the pattern generated by the LED lightning. The LED insect arena system was developed recently and has been widely used for visual stimulation of the fly [31, 32]. We then examined the velocity dependence of the response at different spatial wavelengths (measured in angles as described in Methods) and plotted the response as a function of the temporal frequency of stimulation (rotation speed divided by the spatial wavelength of the stimulus) using either the printed pattern (fig. 2a) or the LED pattern (fig. 2b). Using either type of stimulus, the highest response was obtained in the same range (1–10 Hz) of temporal fre-

Fig. 3. Effect of rearing conditions of CS and *rut* on the optomotor response. **a, b** Visual stimulus contrast dependency of the response in CS flies reared under LD, LL or DD conditions for 4 days (**a**) or 6 days (**b**). The normalized response is shown in flies stimulated with striped paper at 100, 60, 40, 20, 10, or 0% contrast at a temporal frequency of 4 Hz. LD4, *n* = 16; DD4, *n* = 15; LL4, *n* = 13; LD6, *n* = 17; DD6, *n* = 14. Data represent the mean $\pm$ SEM. * *p* < 0.05, ** *p* < 0.01, one-way ANOVA (**a**) and *t* test (**b**). **c** The response to high-contrast (100%) and low-contrast (20%) stimuli in CS and *rut* flies reared under LD or DD conditions for 4 days. Only CS flies reared under DD condition showed the enhancement of response to low-contrast stimuli. CS LD4, *n* = 6; CS DD4, *n* = 8; *rut* LD4, *n* = 7; *rut* DD4, *n* = 9. Data represent the mean $\pm$ SEM. ** *p* < 0.01, *t* test.

quency and was independent of the spatial wavelength (180, 72, 40 or 21° in fig. 2a; 120, 60 or 22° in fig. 2b), demonstrating that the response measured in our system showed this property of Reichardt detectors. In all subsequent studies, a temporal frequency of 4 Hz was used. To further verify the method, we examined another property of Reichardt detectors i.e. the stimulus contrast dependency of the response. Using a black and white striped printed pattern, CS flies clearly showed contrast dependency, since the response increased with increasing pattern contrast and showed clear signs of saturation at high contrast (fig. 2c, left; fig. 3), as shown in previous studies [32, 33]. On the other hand, although the response to the LED striped pattern also depended on stimulus luminance contrast, it showed saturation at lower contrast (fig. 2c, right). The validity of our response quantification method was further confirmed by the finding that the *yw* mutant, which is reported to be motion blind, showed no optomotor response, even at 100% contrast (fig. 2c, bar

on right). Thus, our head-turning angle method is suitable for quantifying the optomotor response and both types of stimulus pattern can be used as the stimulus for the optomotor response. In all subsequent studies, we therefore used the printed pattern stimulus to examine the contrast dependency of the optomotor response in flies reared under different conditions.

The environment in which animals grow often affects their neural responses and behavior. Such plasticity is probably necessary to adapt to changes in environment and is important for survival. To explore whether the neural circuit for the optomotor response in the adult fly brain also showed plasticity, we examined the effect of the light level of the growing environment on the contrast dependency of the optomotor response in CS flies grown in DD for 4 or 6 days after eclosion (DD4 or DD6), on an LD cycle for 4 or 6 days after eclosion (LD4 or LD6), or in constant light for 4 days (LL4). As shown in table 1, there was no significant difference in the average maximum

Table 1. Maximum head-turning angle during the optomotor response

Rearing conditions and stage	Mean maximum head-turning angle (degree)
CS in figure 3a and b	
LD4	11.2±0.4 (n = 16)
DD4	12.0±0.5 (n = 15)
LL4	12.7±0.5 (n = 13)*
LD6	10.5±0.6 (n = 14)
DD6	11.2±0.4 (n = 17)
CS and <i>rut</i> in figure 3c	
CS LD4	12.1±0.9 (n = 8)
CS DD4	12.6±0.9 (n = 6)
<i>rut</i> LL4	8.5±0.4 (n = 10)
<i>rut</i> LD6	9.0±0.5 (n = 7)
CS and DF in figures 4 and 5	
CS LD4	13.0±0.7 (n = 11)
CS LD6	11.1±0.5 (n = 10)
DF LD'4	13.5±0.4 (n = 14)
DF LD'6	12.3±0.7 (n = 11)
DF DD'6	13.0±0.5 (n = 13)

The maximum value of head-turning angle at one time was obtained during all experiments of each fly. Then mean value was calculated. There was no significant difference between flies reared under different conditions at the same developmental stage (t test) besides LL4 (*p < 0.05: significantly different to LD4).

head-turning angle in response to a moving stimulus in flies at the same developmental stage reared under LD and DD conditions, while the average maximum head-turning angle was significantly larger in LL4 compared to LD4 (table 1, Student's t test, $p < 0.05$). However, there were significant differences in the response between rearing conditions (fig. 3a, b; ANOVA; $p < 0.05$). As shown in figure 3a, DD4 flies showed a significantly higher response than LD4 flies to low-contrast stimuli (10 and 20% contrast), but there was no significant difference in response to high-contrast stimuli (40, 60 and 100%). LL4 flies showed a similar contrast dependency with LD4. Interestingly, the plastic change in the optomotor response of DD4 flies was transient, as the effect of the rearing conditions was reduced in flies at 6 days after eclosion. Compared to LD6 flies, DD6 flies showed a significantly higher response to 40% contrast, but no longer showed a higher response to the 10 and 20% low-contrast stimuli, as well as to the 60 and 100% contrast stimuli (fig. 3b). Thus, the neural system required for the optomotor response in the wild-type fly shows a plastic change, but this change is limited and transient. In order to clarify

which signaling pathways are involved in this rearing condition-dependent behavioral change, we examined the optomotor response in *rut* mutants. As shown in figure 3c, CS, but not *rut*, showed the enhancement of the response to 20% contrast stimuli in the dark rearing condition. The response to 100% contrast stimuli was not altered in *rut* in any rearing conditions. There was no significant difference in the average maximum head-turning angle in spite of the rearing conditions, although the angle of *rut* was smaller than that of CS (table 1). These results suggested that the cAMP signaling pathway was important for the behavioral plasticity observed in CS DD4, and our system could be useful to study the molecular mechanism underlying behavioral plasticity.

In the *Drosophila* visual system, 4 days after eclosion is considered to be the critical period [6]. In the above experiment, we demonstrated a plastic change in the optomotor response in CS flies at 4 days after eclosion under DD conditions, but the effect was reversible and lost in later life. To consider whether the plastic change in optomotor response could become fixed in later life and over generations, we examined the optomotor response of the DF, a strain that has been grown in DD since the 1950s at Kyoto University [12, 16]. Although it is likely that DD affects the visual system and it is possible that DFs might have reduced eyesight, they show normal or even increased phototactic behavior [12]. We compared the optomotor response of CS flies tested after 4 or 6 days under LD conditions after eclosion, DFs placed under LD conditions on day 5 after eclosion and tested on day 6 (DD'), and DFs grown on the LD cycle for 2 generations (LD') and tested after 4 or 6 days under LD conditions after eclosion. There were significant differences between rearing conditions (fig. 5; ANOVA; $p < 0.05$). As shown in the results in figure 4 and the summarized results in figure 5, DD' and LD' DFs at 4 or 6 days after eclosion showed a good response to 100% contrast stimulus, which was not different to that of CS flies (6 days) or even better than CS flies (4 days), and the average maximum head-turning angle was not significantly different between flies reared under different conditions at the same developmental stage (table 1). In contrast, DFs at 4 days after eclosion under LD' conditions showed a significantly higher response to a low-contrast (20%) stimulus than CS flies (fig. 5a) and showed an even higher response at 6 days after eclosion (fig. 5b). These results show that DFs do not have reduced visual ability and show an excellent response to low-contrast stimuli. Further, this effect was not lost in DFs reared under LD conditions for 2 generations.

Fig. 4. Head-turning angle of CS and DF flies in response to moving stripes at different levels of contrast. Comparison of the head-turning angle of CS and DF flies in response to stimuli of different contrast (0, 5, 10, 20, 60, 100% contrast) at a temporal frequency of 4 Hz. 0° means the center of the fly's head. Each fly was exposed to clockwise and counterclockwise directions of stimuli, for 10 s each. The rearing conditions were: LD, CS reared on an LD cycle; DD, CS reared in DD after eclosion; DD', DF taken from the dark

room 1 day before the experiment and placed under LD conditions; LD', DF reared under LD conditions for 2 generations before testing. The panels in each line show the mean and SEM for the head-turning angle at different contrasts for CS flies under LD4 conditions ($n = 11$), CS flies under LD6 conditions ($n = 10$), DFs under LD'4 conditions ($n = 14$), DFs under LD'6 conditions ($n = 11$), and DFs under DD'6 conditions ($n = 13$).

Fig. 5. Optomotor response of CS and DF flies to visual stimuli of different contrast at days 4 and 6 after eclosion under the rearing conditions described in figure 4. **a** Visual stimulus contrast dependency of the response to 100, 60, 20, 10, 5, and 0% contrast in flies at 4 days after eclosion (CS LD4, $n = 11$; DF LD'4, $n = 14$). ** $p < 0.01$, t test. **b** Results at 6 days after eclosion (CS LD6, $n = 10$; DF LD'6, $n = 11$; DD'6, $n = 13$). Data represent the mean $\pm$ SEM. ** $p < 0.01$, one-way ANOVA.

Discussion

The optomotor response is a fundamental behavior of most sight-reliant animals. Our findings showed that a new form of behavioral plasticity exists in the visual system of adult flies. This is rather surprising since the de-

velopment and the receptive-field organization of lobular plate tangential neurons, which were high-order visual system neurons and responsible to optomotor response, have been shown to be independent of sensory experience [34, 35]. This plastic change was not observed in *rut*, suggesting that cAMP signaling pathways were important

for this kind of plasticity. Further, we examined the optomotor response in a unique resource, DFs, and found that they showed a good response, especially to low-contrast stimuli. DFs reared under LD conditions for 2 generations showed a similar response, suggesting that their good response is an inheritable property. These results show that the optomotor response in *Drosophila* is a good model system for investigating how rearing conditions affect behavior and how changes are fixed in later life, as well as in the next generation.

We used the head-turning angle to measure the optomotor response and verified it as a quantification method. This method provides an efficient alternative to flight paradigms [18] and is especially useful, since many mutant *Drosophila* do not fly well. For visual stimulation, we used two types of stimuli, a printed stripe pattern and a commercial LED insect arena system [31]. Differences in the optomotor response to different contrast stimuli in CS were more clearly seen using the printed stripe pattern method. However, since the printed patterns depend on the setting of the printer and change with time, we only compared the responses of different flies to the same printed pattern during the same period; in contrast, LED stimulation is highly reproducible at different times and has been widely used for insect visual stimulation. The use of the printed pattern as a visual stimulation was verified by the observation that the responses to both types of stimulation showed the characteristic feature of Reichardt detectors that, for different pattern wavelengths, the response optimum was seen at the same temporal frequency (1–10 Hz) and not at the same image velocity. This optimum frequency was slightly higher than that measured using the neural response (approx. 1 Hz) [28, 32]. A recent report showed that walking modulates the sensitivity of motion vision and that the optimum temporal frequency of neural activity increases with higher motion speeds [36], so it is possible that under our experimental conditions the flies were in an active state, resulting in an increased optimum frequency. Further, the contrast dependency of the response to printed pattern stimulation showed similar saturation nonlinearity to that seen in a previous report [33], although, in response to LED stimulation, the contrast dependency seen in our study was slightly different from that reported for neural activity [32]. The latter discrepancy may also be due to the fly's state or different experimental conditions. However, the response to both printed paper and LED lighting stimuli showed clear signs of saturation at high contrast. These results show that our printed stripe pattern is useful for visual stimulation and that the response quantification

method was verified as an indicator of the optomotor response.

Applying our method to quantify the optomotor response, we found that, in wild-type CS flies the contrast dependency of the response was changed by the rearing conditions, with a higher response to low-contrast stimuli (10–40% contrast) being observed in DD4 flies than in LD4 flies, and this effect was reduced in DD6 flies. Further, dark rearing-dependent plastic change was not observed in *rut* mutants. Mutation of *rut* affects the synthesis of cAMP and has been shown to cause a deficiency in short-term memory and an abnormal synaptic stability and plasticity in neuromuscular junctions [14, 15]. In addition, it has been reported that temperature-dependent synaptic plasticity in neuromuscular junctions was lost in *rut* [37]. Thus, our results indicate that the environment-dependent plasticity we found in CS links to mechanisms controlled by cAMP signaling pathways.

One possible candidate for the cells affected by this change is the L2 cell, a major cell in the lamina. Recently, the L1 and L2 pathways, two parallel pathways at the level of the first relay station in the fly's visual system, have been reported to be important in motion detection in *D. melanogaster* [38]. In this previous report, the L2 pathway was found to be more sensitive to pattern contrast than the L1 pathway. Since L2 cells are known to make feedback synapses onto photoreceptors R1–6, the enhanced contrast sensitivity of L2 cells might have functional significance for these feedback synapses, possibly providing some kind of gain control. Moreover, Kral and Meinertzhagen [10] examined feedback synapses in the housefly, *M. domestica*, and found that the number of L2 feedback synapses depends on the rearing conditions, increasing with dark rearing during the first 4 days of adult life. This plastic change in L2 feedback synapses was reversible and the increase in synaptic frequency was less at 6 days after eclosion, consistent with the transient change in the optomotor response seen in our study. Thus, it is possible that a morphological plastic change in L2 feedback synapses is the cause of the plasticity of the optomotor response seen in our studies, though different species were examined. Not only morphological changes, but also functional plastic changes have been reported in *M. domestica* by measuring electroretinograms, as dark rearing during the first 5 days after eclosion increases light and contrast sensitivity [39]. However, this sensitivity adjustment is not reversible and is present throughout later life. Thus, in addition to L2 feedback synapses, other plastic change(s) may be necessary for functional plasticity in *M. domestica*. Since the receptive-field organization and

development of the high-order visual neurons, lobular plate tangential cells, were reported to be independent on the visual experience [34, 35], irreversible plastic changes may require modifications in these tangential cells together with L2 cells. Moreover, the fact that the changes in the optomotor response in CS seen in our study were reversible may suggest that the number of certain synapses is programmed to decrease later in development. It is therefore important to determine what influences the balance of programmed and nonprogrammed activity-dependent processes during development.

One possible candidate that might influence this balance is prolonged changes in the environment lasting many generations. To investigate this, Mori [11] carried out a long-term experiment in which strains of *D. melanogaster* were maintained over many generations under conditions of complete darkness. These DFs reared in DD for more than several hundred generations show several interesting visual system characteristics, including an increased phototactic response to light and changes in the fine structure of the compound eye. We found that DF, even those reared under LD conditions, showed a higher response to a low-contrast stimulus than wild-type CS flies. This higher response to light would contrast with the situation in animals in caves or in the deep sea, which often lose their eyes, and also to the effect of dark rearing of mammals, which can cause defective visual function [40] in which the number of synapses is likely to decrease with disuse. It has also been reported that *Drosophila* reared in darkness show a decrease in the volume of the lamina in the optic lobe [6]. However, our results showed that visual ability was enhanced by dark rearing. Adaptive plasticity to reared conditions during development was reported in another model organism, the cichlid fish *Aequidens pulcher* [41, 42]. These fish were reared in different spectral environments and then adjusted their vi-

sual system in response to the specific spectral composition to ensure functional color vision in different visual environments. Further, in the nocturnal bee, *Megalopta genalis*, the dendritic fields of the L2 neurons are enlarged, leading to improved dim light vision [43]. A similar mechanism for adaptation to the rearing environment may explain our results for DFs. Genomic alterations induced by dark environment may also be required for adaptation as suggested by a recent report [13]. However, since CS and DF flies have different genetic backgrounds, the ideal control fly, would be one which has the same genetic background as DF and has been reared under LD conditions for the same period as DF. Since it does not currently exist, the best we can do now is to rear DFs under normal LD conditions for many generations and examine whether the response to low-contrast stimuli is reduced.

Evidence is increasing that experience-dependent plasticity does exist in adult invertebrates and this should depend on underlying structural and physiological modifications. It is therefore important to examine the effects of environmental changes on various aspects of function to reveal the molecular mechanism of experience-dependent plasticity and adaptation to environmental changes.

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