A disinhibitory mechanism biases *Drosophila* innate light preference

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Innate preference toward environmental conditions is crucial for animal survival. Although much is known about the neural processing of sensory information, how the aversive or attractive sensory stimulus is transformed through central brain neurons into avoidance or approaching behavior is largely unclear. Here we show that *Drosophila* larval light preference behavior is regulated by a disinhibitory mechanism. In the disinhibitory circuit, a pair of GABAergic neurons exerts tonic inhibition on one pair of contralateral projecting neurons that control larval reorientation behavior. When a larva enters the light area, the reorientation-controlling neurons are disinhibited to allow reorientation to occur as the upstream inhibitory neurons are repressed by light. When the larva exits the light area, the inhibition on the downstream neurons is restored to repress further reorientation and thus prevents the larva from re-entering the light area. We suggest that disinhibition may serve as a common neural mechanism for animal innate preference behavior.
When choosing between two alternative conditions, animals such as Drosophila larva reorientates when facing unfavorable conditions but maintain unchanged directions when facing favorable conditions. The choice behavior involves transforming sensory input into motor action of reorientation. In vertebrates, although the brain regions or neurons that are responsible for sensory information processing and motor control have been relatively well mapped, the cellular and molecular mechanism underlying sensorimotor transformation in central brain has only been reported in a few cases, such as cutaneous or olfactory input-induced locomotion in xenopus and lamprey.

In Drosophila larva, Bolwig’s organs, i.e. the photoreceptors, regulate larval avoidance response to light in both laboratory and outdoor experiments. Bolwig’s organs directly send projections into the larval optic neuropil (LON) in central brain and synapse on visual local neurons and visual projection neurons. These downstream neurons, including visual local neurons such as IOOLP (local optic lobe pioneer) neurons, and visual projection neurons specified as pdf neurons and 5th lateral neuron in clock circuit as well as PVLO9 neurons (posterior ventro-lateral neuron 09), have been reported to be involved in various forms of larval light navigational behaviors. At the level of motor control, neurons in Drosophila larval SEZ (subesophageal zone) have been suggested to command larval reorientation behavior in light avoidance. But the neuronal circuitry that bridges the gap between upstream visual processing neurons and downstream turning command neurons has been left blank. How the visual signal is transformed into an avoidance behavior remains largely elusive.

Disinhibition is a central mechanism that serves in various neural functions, such as sensory signal processing, selection of motor programs, memory expression, and the switch between wake and sleep status. In a disinhibitory microcircuit, the inhibition on downstream inhibitory neurons is supposed to be tonic, whereas the inhibition on upstream inhibitory neurons should be phasic. This enables the efficient temporal control of the excitability of the downstream neurons.

Here we show that Drosophila larval avoidance to light is gated in a disinhibitory manner. We propose that disinhibition is the underlying mechanism for the initiation of choice action and subsequent securing of the correct choice in animal choice behavior.

Results

Larval light avoidance requires inhibition of LRINR13B07s. To discover neurons that inhibit Drosophila larval reorientation in light avoidance, we crossed Gal4 lines with UAS-NaChBac which increases neuronal excitability and tested the larvae in a light/dark choice assay at a light intensity of 500 lux (23.3 μW/mm²). One Gal4 line of R13B07-Gal4 that labeled about seven to ten interneurons in the anterior part of each larval brain hemisphere and a group of neurons in posterior part of VNC (ventral nerve cord), in addition to the Rh6-positive photoreceptors in peripheral nervous system, demonstrated an abolished larval light avoidance (Fig. 1a, b, Supplementary Fig. 1a–c). This defect was not rescued by introduction of tsh-Gal80 that specifically represses Gal4 activity in VNC, suggesting that larval light avoidance did not involve the R13B07-Gal4 labeled neurons in the VNC (Fig. 1a, Supplementary Fig. 1a–c). As hyperactivating Rh6-positive neurons alone did not affect larval light avoidance, they could also be excluded (Supplementary Fig. 2d). So it should be the neurons in brain hemispheres that repressed larval light avoidance.

As activation of these neurons negatively regulated light avoidance, we hypothesized that these neurons might be inhibited by light. We then tested the responses of the brain hemisphere neurons to light in calcium imaging. Among these neurons, only one pair of antero-laterally localized neurons that each possessed a comprehensive dendritic region posterior-medial to the cell body and a dense sytGFP labeled axonal arborization region anterior-medial to the dendrites (Fig. 1c, Supplementary Fig. 1e, f), was strongly inhibited by blue light stimulation (Fig. 1d, e). As these neurons turned out to be inhibitory later, we named these R13B07-Gal4 labeled light repressed inhibitory neurons LRINR13B07s. The inhibition persisted as light was on for up to 3 min (Fig. 1f). When light intensity increased, this inhibition was strengthened (Fig. 1g). As the dendritic regions of the neurons were adjacent to the axonal projection area of lateral clock neurons outlined by anti-PDF, we reasoned that they might receive visual inputs from the clock neurons (Fig. 1e, 1i). We made use of a RASD05-LexA line that was generating using a fragment of the promoter of clock (clk) gene. This line labeled pdf neurons and neurons that were morphologically similar to the fifth lateral neurons (5th LN), DN1 (dorsal neuron 1), DN2 (dorsal neuron 2), in addition to three clusters of putative immature neurons in each brain hemisphere (Supplementary Fig. 3). When the clk-LexA labeled neurons were blocked with TNTG, a presynaptic inhibitor of neurotransmission, the extent of the inhibition was significantly reduced, suggesting that the inhibition at least partially originated from the clk-LexA labeled neurons (Fig. 1h). Specifically ablating pdf neurons using pdf-DTI (DTI, diphertheria toxin) could also relieve the light-induced inhibition, but obviously not as effective as inhibiting clk-LexA neurons (Fig. 1h). We next asked which receptor was used by LRINR13B07s to receive the inhibitory input. Knocking down in LRINR13B07s the expression of a GABA/Glycine receptor GRD27,28, but not of another GABA receptor RDL29, could efficiently reduce the extent of inhibition (Fig. 1i). This meant that the inhibition of LRINR13B07s by light was mediated by the GRD receptor.

LRINR13B07s are GABAergic. As the LRINR13B07s were inhibited by light and the activation of LRINR13B07s suppressed larval light avoidance, we reasoned that these light inhibited neurons might inhibit other neurons that promote light avoidance. We co-stained the antibody against GABA, the most widely used inhibitory neurotransmitter, with R13B07-Gal4 to test whether they were indeed inhibitory. The anti-GABA signal indeed co-localized well with the cell bodies of the LRINR13B07s (Fig. 1j, k). LRINR13B07s signal did not co-localize with anti-ChAT (choline acetyltransferase) that marks the cholinergic neurons, but about three other R13B07-Gal4 labeled neurons in each larval brain hemisphere did (Supplementary Fig. 1g–i). This was verified by our observation that introduction of Cha-Gal80 reduced the number of R13B07-Gal4 labeled neurons by about three in each brain hemisphere (Supplementary Fig. 1j). The remaining labeling of axonal termini of photoreceptor neurons might be due to insufficient repression on Gal4 activity by Cha-Gal80. On the other hand, vGlut-Gal80, which was assumed to be expressed in glutamatergic neurons, did not obviously affect the expression of R13B07-Gal4 in larval brain (Supplementary Fig. 1k). We then tested the role of GABA in LRINR13B07s in larval light avoidance using the light/dark choice assay. When we knocked down expression of GABA synthesizing enzyme GAD (glutamate decarboxylase) or vesicular GABA transporter vGAT (vesicular GABA transporter) in the LRINR13B07s, larval light avoidance was enhanced (Fig. 1l). It should be noted that we used relatively weaker light of 250 lux (11.7 μW/mm²) instead of 550 lux (23.3 μW/mm²) in the assay to reduce the level of light avoidance in control lines, thus making more space for further improvement in light preference index. Although the possibility that LRINR13B07s
were cholinergic or glutamatergic could not be completely excluded based on absence of labeling, they were the only R13B07-Gal4 labeled neurons that were GABAergic and inhibited by light. Therefore, they should be the neurons that were responsible for larval light avoidance among all the R13B07-Gal4 labeled neurons. As LRIN\textsuperscript{R13B07} were inhibitory neurons that were inhibited by light, larval light avoidance thus might be regulated by a disinhibitory mechanism\textsuperscript{18}–\textsuperscript{23}.

\textbf{LRIN\textsuperscript{R13B07} inhibit CLPN\textsuperscript{R82B09}.} We next searched for the light avoidance promoting neurons downstream of LRIN\textsuperscript{R13B07}. Inhibition of the LRIN\textsuperscript{R13B07} using an optogenetic tool NpHR induced a robust calcium signal increase in one pair of central brain neurons labeled by R82B09-Gal4 (Fig. 2a–c), suggesting that these neurons had been subjected to tonic inhibition from the LRIN\textsuperscript{R13B07}. These contralateral projecting neurons were then named as CLPN\textsuperscript{R82B09}. They were also labeled by R82B09-LexA and R82B10-Gal4 (Supplementary Fig. 4a–c). The CLPN\textsuperscript{R82B09} had a small axonal arborization region in the medial contralateral brain hemisphere, a widespread dendritic region near the dendrites of the LRIN\textsuperscript{R13B07}, and a smaller dendritic arborization area in larval SEZ (Fig. 2d, Supplementary Fig. 4d–f). The dendrites of LRIN\textsuperscript{R13B07} were found to be overlapping with the dendrites of the CLPN\textsuperscript{R82B09} (Fig. 2e). We then used GRASP technique to confirm the contact between LRIN\textsuperscript{R13B07} and...
CLPNR82B09s. A strong GRASP signal was seen in the overlapping region (Fig. 2f, Supplementary Fig. 5). The putative synaptic contact was validated by trans-Tango30, a newly developed technique that can be used to probe downstream synaptic partners of neurons. By driving expression of trans-Tango with R13B07-Gal4 to search for immediately downstream neurons, a large amount of cells were successfully marked (Supplementary Fig. 6a–b). Some of these cells were in the same region of CLPNR82B09s (Supplementary Fig. 6b). We then added R82B09-Gal4 in the system to see if the trans-Tango signals driven by R13B07-Gal4 overlap with the GFP signals that marked CLPNR82B09s. As expected, co-localization was readily found (Fig. 2g–i). This result was in support of a direct dendrodendritic interaction between LRINR13B07s and CLPNR82B09s, as driving trans-Tango using R82B09-Gal4 alone did not yield any CLPNR82B09 signal (Supplementary Fig. 6c, d). As LRINR13B07s were GABAergic (Supplementary Fig. 6c), we reasoned that CLPNR82B09s should be subjected to direct GABAergic inhibition.

To confirm this hypothesis, we applied drugs to dissected and digested larval brain samples, in which CLPNR82B09s were dissociated and more susceptible to drug application. GABA could efficiently repress the excitation of CLPNR82B09s induced by acetylecholine in calcium imaging (Fig. 2j, k). This result meant that CLPNR82B09s indeed subjected to GABAergic inhibition, in addition to cholinergic excitation. Furthermore, the application of RDL antagonist picrotoxin could strongly activate CLPNR82B09s (Fig. 2l, m), while RDL agonist etomidate could efficiently inhibit CLPNR82B09s (Fig. 2n, o). Together, these data suggested that CLPNR82B09s used RDL to receive GABAergic inhibitory input from LRINR13B07s.

Light responsive CLPNR82B09s control larval reorientation. We next examined if CLPNR82B09s were required for larval light avoidance. Blocking CLPNR82B09s by expression TNGT with either R82B09-Gal4 or R82B10-Gal4 abolished larval preference for darkness in the light/dark choice assay at 550 lux (23.3 μW/mm²) (Fig. 3a). Introduction of Cha-Gal80 which removed Gal4 activity in all neurons except CLPNR82B09s did not rescue the defect (Fig. 3a and Supplementary Fig. 4c). Additionally, knocking down RDL expression in CLPNR82B09s enhanced the larval preference for darkness over light when a relatively weaker light intensity of 250 lux (11.7 μW/mm²) was used (Fig. 3b). On the other hand, we expressed UAS-Chrimson with R82B10-Gal4 to activate the CLPNR82B09s, robust larval head casts were observed (Fig. 3c, d). This could even be realized by photogenetic activation of a single CLPNR82B09 (Supplementary Video 1). These results suggested that the CLPNR82B09s might control light-induced head cast. Indeed, our calcium imaging results showed that the CLPNR82B09s did respond to light stimulation (Fig. 3e, f). It is noted that the calcium transient could last for up to 40 s, whereas larval light avoidance response generally takes only at most a few seconds. The long duration of calcium response was likely due to dissection of larval body before calcium imaging that greatly changed the physiological environment of the imaged neurons. Knocking down RDL expression in the CLPNR82B09s not only increased the probability (see Methods for more details) (Fig. 3g), but also significantly improved the amplitude of the response (Fig. 3e, f). As a neuronal calcium transient usually reflects the accumulative effect of a bout of action potentials31–33, these results were in consistence with the hypothesis that CLPNR82B09s did respond to light or not, but not how strong the responses were. The above results together suggested that CLPNR82B09s controlled the light-induced head cast in light avoidance.

Disinhibition of CLPNR82B09s facilitates larval head cast. Despite that light inhibits LRINR13B07s and LRINR13B07s inhibit CLPNR82B09s, it was still not clear if the light-induced reorientation was regulated by disinhibition of CLPNR82B09s through inhibition of LRINR13B07s. To confirm this hypothesis, we first tested whether the CLPNR82B09s’ response to light was regulated by the LRINR13B07s-CLPNR82B09s disinhibition. Knocking down GRD expression in LRINR13B07s to reduce the light-induced inhibition on LRINR13B07s could significantly reduce the probability for CLPNR82B09s to respond to light, while the amplitude of the response seemed to be unaffected (Fig. 4a and Supplementary Fig. 8). On the other hand, when we knocked down GAD expression in LRINR13B07s to relieve the GABAergic inhibition on CLPNR82B09s, the probability for CLPNR82B09s to respond to light was significantly improved, although the amplitude of the response was also unaffected (Fig. 4a and Supplementary Fig. 8). These results were in consistence with the effects of blocking clk-LexA neurons as shown in Fig. 3f, g. It was probably because blockage of clk-LexA neurons reduced light...
inhibition on LRIN\textsuperscript{R13B07} that CLPN\textsuperscript{R82B09} were no longer efficiently disinhibited. In the case of ablating pdf neurons, light inhibition on LRIN\textsuperscript{R13B07} was also reduced but not as much as blocking \textit{clk}-\textit{LexA} neurons, so that the inhibition of CLPN\textsuperscript{R82B09} by LRIN\textsuperscript{R13B07} could still be relieved. Taken together, these results suggested that CLPN\textsuperscript{R82B09}'s response to light was indeed gated by inhibition of LRIN\textsuperscript{R13B07}.

We next tested whether light-induced larval head cast was also regulated by the disinhibitory mechanism. We measured size of larval head cast in response to light-on using a light spot assay under a dim white light as larval head cast size saturated when light became perceivably high. We used relatively higher (26.34 pW/mm\textsuperscript{2}, measured at 470 nm, see Methods) or lower (1.80 pW/mm\textsuperscript{2}, measured at 470 nm) light intensity to elevate or lower the...
level of head cast size in controls, so as to make enough room for further decrease or increase in experimental groups. We first knocked down expression of GRD in the LRINR13B07s to mitigate the inhibition of LRINR13B07s by light. As shown in Fig. 4b, size of larval head cast upon light-on was significantly reduced in the GRD knockdown group as compared to that of the controls, at light intensity of 1.80 ± 0.05 pW/mm². This suggests that inhibition of the inhibitory LRINR13B07s was indeed necessary for larval aversive response to light. On the other hand at relatively weaker light intensity of 1.80 ± 0.05 pW/mm², when GAD or vGAT was downregulated in LRINR13B07s, the size of light-induced larval head casts was significantly enhanced (Fig. 4c). In addition, knocking down RDL expression in CLPNR82B09s also significantly enhanced size of larval head cast upon light-on at light intensity of 1.80 ± 0.05 pW/mm² (Fig. 4d). These results were consistent with our previous conclusion that disinhibition of CLPNR82B09s through inhibiting LRINR13B07s facilitated CLPNR82B09s response to light since CLPNR82B09s firing probability is positively related to the ratio of light-induced head cast over spontaneous head cast. As sizes of the light-induced head casts are usually larger than that of the spontaneous ones, CLPNR82B09s firing probability is positively related to the measured head cast size.

Next, to exclude the possibility that the increased head cast sizes resulted from the manipulations of neuronal activities even in absence of light, we performed the light spot assay with light constantly kept off, i.e. the light spot was actually dark. The differences in larval head cast in response to “light-on” were no longer observed (Supplementary Fig. 9a, b). Thus, the increased head cast sizes were indeed the outcomes of the interaction between the neuronal activities and light stimulation. Taken together, larval head cast response to light-on was indeed facilitated by disinhibition of CLPNR82B09s via LRINR13B07s.

Re-inhibition on CLPNR82B09s represses larval head cast. Because light inhibition on LRINR13B07s was removed after the light was turned off (Fig. 1f), we speculated that the inhibition of LRINR13B07s on CLPNR82B09s would be naturally restored once the larva had exited the light spot. Further head cast would therefore be inhibited. We then examined the role of LRINR13B07s-CLPNR82B09s inhibition in head cast for larvae that had just exited the light spot. As expected, at relatively high light intensity of 26.34 ± 0.05 pW/mm², knocking down the light receiving receptor GRD in LRINR13B07s did not affect larval head cast size upon light exit as the absence of GRD did not affect LRINR13B07s-CLPNR82B09s inhibition in darkness (Fig. 5a). However, knocking down GAD or vGAT expression in the LRINR13B07s could significantly improve size of head cast upon light exit (Fig. 5b), as could also be seen in the larvae with RLDN downregulated in the CLPNR82B09s (Fig. 5c), at light intensity of 1.80 ± 0.05 pW/mm². The enhancement in larval head cast upon “light exit” was not seen if light was constantly kept off during the assay (Supplementary Fig. 9c, d). These results meant that the stimulatory effect of light on larval head cast could persist even after light went off. The immediate restoration of the inhibition on CLPNR82B09s prevented further head cast upon light exit.

As head cast could potentially bring the larva back into the light spot after it had left, the immediate restoration of the LRINR13B07s-CLPNR82B09s inhibition might help to secure the “correct” choice of light escape by repressing the potential larval head cast. We then tested this assumption by examining the chance for the larva to return to light spot after the initial light escape. Knocking down GAD expression in LRINR13B07s to prevent restoration of LRINR13B07s-CLPNR82B09s inhibition indeed significantly increased the rate of larvae returning to light spot from no more than 15.00 ± 39.02% (Fig. 5d, Supplementary Fig. 10). Similarly, knocking down RDL in the CLPNR82B09s also increased the rate from 8.33 ± 39.67% (Fig. 5e). Thus, the restoration of inhibition on CLPNR82B09s could help improve larval light avoidance by repressing potential improper head cast after the initial light escape.

Discussion

In this work, we discovered a disinhibitory neural mechanism that gated Drosophila larval head cast in presence and absence of light (Fig. 5f). LRINR13B07s that exert a tonic inhibition on the larval head cast controlling CLPNR82B09s are inhibited by light to facilitate the head cast response. Once the larva escapes from light successfully, the inhibition on CLPNR82B09s is naturally restored to prevent further improper head casts, thus securing the success of light avoidance.

Such a disinhibitory mechanism has several roles. First, the inhibition on CLPNR82B09s represses larval behavioral response to light. This justifies the reduced larval responsiveness to very dim light that is usually safe. It also helps larvae to terminate behavioral response to light when necessary. Second, the inhibition of LRINR13B07s by light ensures the specificity of larval orientation in response to light, as the inhibition on CLPNR82B09s prevents their excitation by non-visual stimulus. This specificity can be further enhanced if there exists another pathway for light to stimulate CLPNR82B09s. Third, the opposite regulation of reorientation at transitions between light and darkness enhances larval preference for darkness over light. In addition to the explicit light avoidance facilitated by the head cast upon light stimulation, light avoidance is also enhanced by the inhibition of further potential head casts after light escape, in an implicit manner.
Compared with the recently reconstructed larval visual system that includes the first to third order neurons\textsuperscript{13}, LRIN\textsuperscript{R82B09}s and CLPNR82B09s should be at least the 3rd or higher order neurons. For the known reconstructed second order visual neurons that likely project to region of LRIN\textsuperscript{R82B09}s dendrites, all are cholinergic except for pdf neurons\textsuperscript{13}. As pdf neurons carry only part of the inhibitory signal to LRIN\textsuperscript{R13B07}s, those cholinergic neurons must activate some downstream inhibitory neurons that inhibit LRIN\textsuperscript{R13B07}s. pdf neurons themselves may also inhibit LRIN\textsuperscript{R13B07}s through other downstream neurons. Besides the

\textbf{Fig. 3} The CLPN\textsuperscript{R82B09}s control head cast response to light. \textbf{a} Inhibiting CLPN\textsuperscript{R82B09}s abolishes larval avoidance to white light at 550 lux (23.3 \text{mW/mm}\textsuperscript{2}). \textbf{b} Knocking down RDL expression in CLPN\textsuperscript{R82B09}s enhances larval avoidance to white light at 250 lux (11.7 \text{mW/mm}\textsuperscript{2}). \textbf{c}\textbf{d} Optogenetic stimulation of CLPN\textsuperscript{R82B09}s evokes larval head cast. \textbf{d} is the statistics of head cast sizes in \textbf{c}. The pink bar indicates the period of optogenetic stimulation. \textbf{e}\textbf{g} Knocking down RDL expression in CLPN\textsuperscript{R82B09}s increases both amplitude and probability of CLPN\textsuperscript{R82B09}s’ response to light in calcium imaging. The blue bar in \textbf{e} indicates 470 nm light stimulation at intensity of 10.58 \text{mW/mm}\textsuperscript{2}. \textbf{f} is the statistics of peak responses in \textbf{e}. \textbf{h}–\textbf{i} Blocking clk-LexA neurons with TNTG does not affect the amplitude (\textbf{h}), but undermines the probability of CLPN\textsuperscript{R82B09}s’ response to light in calcium imaging (\textbf{i}). One second 470 nm light stimulation at intensity of 10.58 \text{mW/mm}\textsuperscript{2} was used. In \textbf{e}\textbf{–}\textbf{i}, R82B09>GCAMP6m is the short for UAS-GCAMP6m/R82B09-Gal4. R82B09>GCAMP6m+RDL-RNAi is the short for UAS-GCAMP6m/R82B09-Gal4; R82B09>GCAMP6m is the short for UAS-GCAMP6m; R82B09-Gal4 is the short for UAS-GCAMP6m/R82B09-Gal4. Numbers above columns in \textbf{g} and \textbf{i} indicate sample sizes. n.s. not significant, *P < 0.05, **P < 0.01, ***P < 0.001, one-way ANOVA with Tukey’s post hoc test in \textbf{a}\textbf{–}\textbf{b}, \textbf{d}, and \textbf{f}, t-test in \textbf{h}, fisher’s exact test in \textbf{g} and \textbf{i}. Error bars, SEMs. Source data of \textbf{a}\textbf{–}\textbf{i} are provided as a source data file.
second order neurons, one pair of third order glutamatergic visual projection local neurons also project to the region of LRINR13B07’s dendrites. They may channel part of the inhibition on LRINR13B07. As for CLPNR82B09, they are disinhibited by light through the disinhibitory pathway. As they are also susceptible to cholinergic excitatory input (Fig. 2), it is possible that CLPNR82B09 can also be activated by light through cholinergic neurons. On the output side, as parts of dendritic termini of CLPNR82B09 end in SEZ, the region that has been suggested to be crucial for motor control15,16,35, CLPNR82B09 may thus target onto the turning command neurons in SEZ to regulate larval head cast. It is likely that CLPNR82B09 also control larval head cast response to aversive sensory stimuli in other modalities, such as gustation or olfaction.

The observation that pdf neurons channel part of the light inhibition on LRINR13B07’s prompts us to reconsider the role of pdf neurons in larval light avoidance. It has been well established that light could entrain larval clock and induce photophobic behavior through Bolwig’s organs. Downstream of Bolwig’s organs, pdf neurons were known to mediate the entraining of clock, but their roles in light avoidance has been on debate7,12. Based on our observation, pdf neurons do have the potential to affect larval light avoidance through mediating the light inhibition on LRINR13B07, although ablating pdf neurons did not significantly change CLPNR82B09’s response to light. It is possible that their role in light avoidance cannot be readily detected, unless light signal goes through those non-pdf neurons is lessened.

One important property of LRINR13B07 in the disinhibitory neural circuit is that it is sensitive to dim light, although the light inhibition does not saturate even at high light intensities. But at behavioral level, the size of the disinhibition regulated larval head cast in response to dark-to-light transition seemed to saturate at moderate light intensity. Therefore, when larvae choose a dark or dim condition over a brighter condition, the light avoidance must be involved. If it is not, the disinhibition of CPLNs makes an almost completely dark local environment. On the other hand, when larvae choose between bright and brighter light, light Avoidance may not happen. If it does, the disinhibition of CLPNs should not be involved.

The disinhibitory control of light elicited larval turning behavior resembles the disinhibitory mechanism in vertebrates for motor program selection. In vertebrate, striatal neurons from motor cortex send inhibitory signal to inhibit pallidum neurons that exert tonic inhibition on spinal motor command neurons20. One advantage of this mechanism is the strict and precise control...
of motor initiation and termination. The adoption of disinhibitory mechanism in Drosophila larval motor control suggests that the higher level control of motor initiation is conserved among invertebrate and vertebrate.

As most innate preferences are realized through reorientation, we suggest disinhibition to be a common neural mechanism underlying animal innate preference behavior.

Methods

Fly culture and strains. All flies were raised at 25 °C on standard medium and 12 h:12 h light/dark cycles of culture 66. The following fly strains were used in this work: w1118, Rhs-eGFP(BL6600), Rhs-eGFP(BL7461), R66-Gal4(BL7464), GMR-myrGFP(BL7211), R13B07-Gal4(BL48545), R28B10-Gal4(BL46717), R28B09-Gal4(BL40133), R28B09-LexA(BL61613), cik-LexA(R43D05-LexA,BL54147), pdf-LexA 67, Cha-Gal80, vGut-Gal80, Ish-Gal80, GMR-Gal80, UAS-TNTG 69, UAS-NavC 70, UAS-GCAMPtrm(BL2748), UAS-mCD8-GFP(BL5137), UAS-myrGFP(BL7118), UAS-Chrimson(BL5135), UAS-NpHR(BL1753), UAS-sytGFP, UAS-CD4::GFP11;UAS-CD4::GFP1-10, UAS-GrD-RNAi(TH02214.N), UAS-vGAT-RNAi(THU4304), UAS-RDL-RNAi(TH02821.

Light spot assay. The procedure of light spot assay was largely same as previously described 46. Individual larva was first acclimated on an agar plate for 2 min in a dark room. The plate was rotated to re-orientate larva heading toward a light spot of 2 cm-in-diameter generated by a white light LED before start of test. The white light had one intensity peak at 450 nm with half width of 10 nm and another peak at 585 nm with half width of 65 nm. The light intensity of 1.80 pW/mm² or 26.34 pW/mm² measured at 470 nm was used. Experimental temperature was kept as 25.5 °C. Whole process from larval entering to exiting of the light spot was video recorded with an infrared high-resolution web camera from above. The lens was covered by a 850 nm infrared narrow-pass filter to prevent the disturbance of visible light on video. Three 850 nm LED lights that were evenly placed around the plate to illuminate the arena. Experimental temperature was kept as 25.5 °C. Videos were analyzed with the software SOS and custom scripts (see below for larval head cast analysis). For larval re-entry to light spot, a larva that had its head touched the light spot for at least once within 20 s after the initial exit was defined as a larva of returning.

Optogenetics. Eggs of proper genotypes were laid on food supplied with 0.2 mM trans-retinal. For testing behavioral consequence of optogenetic activation of CLPNR82B09s with Chrimson, cleaned single 3rd instar larva was allowed to crawl on a 1.5% agar plate. A 620 nm red-light pulse was delivered onto the larva in periods of straight forward locomotion. The process of larval locomotion was video recorded and processed with SOS software and custom scripts (see below for larval head cast analysis). For imaging CLPNR82B09s response to optogenetic inhibition of LRINR13B07 using NpHR, 540 nm green light was used (also see below for calcium imaging).
Pharmacology. Larval brain samples were prepared following previously reported protocols but with modifications35. Individual 3rd instar larva was removed from food and washed alternatively with ddH2O and 70% ethanol for 1 min. It was dissected in standard saline solution (128 mM NaCl, 2 mM KCl, 4 mM MgCl2, 1.8 mM CaCl2, 36 mM sucrose, 5 mM HEPES, pH = 7.1) and the brain was transferred to 1.5 ml microcentrifuge tube with 500 μl standard saline containing 0.4 mg/ml protease (Sigma-Aldrich, F5147) and 0.1 mg/ml collagenase (Sigma-Aldrich, C0130). The brain was digested for ~3 hr before being centrifuged at 1 rcf for 2.5 min. The digest solution was pipetted off and 100 μl Schneider’s Drosophila Medium (Gibco, 21720-024) containing 10% FBS (Sera Pro, S601S-500) was added. Single brain was transferred into round shaped recording chamber (16 mm diameter) filled with 1.5 ml standard saline for calcium imaging. To prevent the sample from moving during circulating, brain tissue was covered by a custom made stainless steel grids (mesh size 200, hole size 100 μm). During calcium imaging, drugs were added into the recording chamber using a circulating pump (LongerPump, BT100-J). Drugs used included picrotoxin (Hellobio, HB0506), etomidate (Mechem Express, HY-B0100), GABA (Sigma-Aldrich, A5835) and acetylcholine chloride (Sigma-Aldrich, P6625).

Calcium imaging. Calcium imaging was similar to previous report36. Individual clean 3rd instar larva was briefly dissected in AHI (Adult Hemolymph-like) solution to expose central brain, but with the anterior part of body intact. It was then transferred with AHI solution into a chamber formed by reinforcing rings on a glass slide and covered with a cover slip. The target neuron was directly localized by continuously making inferences about its position. Headtheta, was calculated from the angle between head-midpoint line and midpoint-tail line. The angular speed of headtheta is termed headomega.

Immunohistochemistry. Body, headtheta, was calculated from the angle between head-midpoint line and midpoint-tail line. The angular speed of headtheta is termed headomega.

Immunohistochemistry and confocal microscopy. Third instar larval brains were dissected from larvae in PBS, fixed in PBS containing 4% paraformaldehyde for 1 h at room temperature and washed 4 × 30 min in PBS containing 0.5% Triton-X 100 (PBT) before being blocked for 2 h in PBT containing 5% goy saerum. The samples were then incubated with primary antibodies (rabbit anti-GABA, 1:50, Cat. A2052, Sigma-Aldrich; rabbit anti-RFP 1:100, Cat. ab26341, Abcam; mouse anti-rCD2, 1:100, Cat. 203130, Biologend; rabbit anti-CD4, 1:200, Cat. ab133616, Abcam; mouse anti-PDF, 1:100, PDF-C7 concentrate, DSHB; mouse anti-Fas II, 1:100, 1D4 concentrate, DSHB; mouse anti-ChAT, 1:100, ChATB41 concentrate, DSHB; rat anti-HA, 1:200, Cat. 11867423001, Roche) overnight at 4 °C, before being washed in PBT for 4 × 30 min. Specifically, as QUAS-mtdTomato(3xHA) was the designated reporter for trans-Tango signal, we used anti-HA to visualize the trans-Tango signal. The samples were then incubated with secondary antibody (Alexa 647-conjugated goat anti-rabbit, 1:100, Cat. A27040, Alexa 647-conjugated goat anti-mouse, 1:100, Cat. A21235, Alexa 647-conjugated goat anti-rat, 1:100, Cat. A21247, or Dylight 594-conjugated goat anti-rat, 1:100, Cat. SA5-10020, all from Thermo Fisher) for 2 h at room temperature and washed in PBT 3 × 10 min in darkness before being mounted and viewed. Images were acquired using an Olympus FV-1000 confocal laser scanning microscope and subsequently processed with ImageJ (www.nih.gov/ij). Specifically for processing the images about GRASP between LINR[N1380] and CLPN[R2809], the GRASP signal was confirmed by continuously tracing the anti-CD4 signals that marked the morphology of LINR[N1380] and CLPN[R2809], so that they were separated from the non-specific GFP signals.

Larval head cast analysis. The larval behavioral details in light spot assay were analyzed with a modified version of SOS36. In brief, single larva was sketched out from background and thinned to a line by algorithms implanted in matlab (Mathworks Inc.). The two ends of the line, head, and tail, together with midpoint of the line and centroid obtained from the larval body outline, were used to calculate the headspeed, tailspeed, midspeed, and centroid. The bending of larval body, headtheta, was calculated from the angle between head-midpoint line and midpoint-tail line. The angular speed of headtheta is termed headomega.

For larval head cast size upon light-on and light-off in light spot assay, the size of head cast upon light-on was defined as previously described with modifications35. As larva head cast happened always after deceleration, they were considered as an assembly when we judged the light related events. First, periods in tailspeed was defined according to the fluctuation of tailspeed during larval peristalsis. Deceleration was defined if the minimum tailspeed in one period was lower than in the previous period for more than 15% and the maximum tailspeed was no higher than in the previous period. Multiple continuous deceleration periods joined into one deceleration segment. Second, the time window for picking the deceleration related head cast was defined in either of the following ways: if larval tailspeed dropped to a level below arbitrarily set threshold, the time window was the whole subthreshold period; if the larval tailspeed after deceleration was above the threshold, the time window was from 1 tailspeed period before to 2 tailspeed periods after the end of the deceleration. Third, the largest head cast size in the time window that began within 2 tailspeed periods before or 4 tailspeed periods after larva entered light spot was picked as the size of head cast upon light-on. For the largest size upon light-off, the larval head cast size in the time window of 0.5–5 s after larva exit the light spot, or in the time window from 0.5 s after light exit to the re-entry of light if the re-entry was within 5 s after light exit, was picked as the size of head cast upon light off. The extracted values of head cast sizes were confirmed by reviewing the videos. Head cast data that did not match with the videos due to improper image processing were discarded.

Size of larval head cast in response to optogenetic stimulation of CLPN[R2809] was extracted in a similar way as in response to light-on in light spot assay, except that a 5 s time window for measuring head cast size was set as from the beginning to 4 s after the ending of the 1 s red light stimulation.

The codes are available at http://www.github.com/zfgong/zpp.

Statistics. Statistics was performed with prinsm6.0 (Graphpad Inc.). Fisher’s exact test was used for comparing the rate of event occurrence between two groups. For comparing of event occurrence rate among three groups, we made use of the companion package of R (version 3.5.0) and did p-test. The p-values between subgroup comparisons were adjusted using FDR (false discovery rate) controlled by the Benjamini-Hochberg method. For all the rest, t-test or one-way ANOVA with Tukey’s post hoc test were used. Error bars in scatter plot and shaded areas flanking curves represented SEM. The details of the statistics for relevant figures panels are in Supplementary Summary of Statistics.

Data availability. The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files. The source data underlying all plotted Figures and Supplementary Figures are provided as a Source Data file.

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**Additional information**

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