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Activity-dependent synaptic plasticity through regulation of histamine receptor in the *Drosophila* visual system

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Contents

Abbreviations

List of Abbreviations	Definition
AMPAR	AMPA glutamate receptor
AZ	Active Zone
BAC	chromosome
Brp	Brunchpilot
CaMKII	Calmodulin kinase II
CREB	cAMP response element-binding protein
DD	continuous dark condition
EE	environmental enrichment
FLP	flippase
FRT	flippase recognition target
GRASP	GFP Reconstitution Across Synaptic Partner
HR	histamine receptor
LD	normal light-dark condition
LL	continuous light condition/constant light exposure
LNv	ventral lateral neuron
LTD	long-term depression
LTP	long-term potentiation
NMDAR	NMDA receptor
OLLAS	OmpF Linker and mouse Langerin fusion Sequence
PBS	phosphate buffer saline
SA	splice acceptor
TRIC	Transcriptional Reporter of Intracellular Calcium
TRPA1	Transient receptor potential cation channel A1
UAS	upstream activation sequence

Abstract

'Plasticity' allows animals to develop mature brains, recover from injury, and respond to various stimuli and experiences from the environment through modifying the structure, connection, or molecular process in their brains. The plasticity occurring during adaption happens in different scales, including cellular scale and subcellular scale. On a subcellular scale, plasticity occurs at the sites where neurons make connections with each other known as synapses. Synapses allow one neuron (presynaptic neuron) to pass a chemical or electrical signal to another (postsynaptic neuron). For chemical synapses, presynaptic neurons will release chemical substances called neurotransmitters which are contained in synaptic vesicles. Activity-dependent synaptic plasticity is believed to be critical for responses to the environment and adaptive behaviors. When a circuit receives input activity, direct synaptic changes occur and have subsequent consequences in the circuit. It still requires more studies on how synaptic plasticity affects the circuit change. One type of presynaptic neuron usually has connections with various types of postsynaptic neurons. It remains a big question whether synaptic plasticity happens among all the connections, or only some specific subsets of postsynaptic neurons change the connections with the presynaptic neuron and modify their activity responses according to input activity.

For the activity-dependent plasticity research at the synaptic level, plenty of genetic tools and experimental methods have been developed in model animal *Drosophila Melanogaster*, which is also called 'fruit fly'. The *Drosophila* visual system is a powerful model to study the activity-dependent synaptic plasticity invoked by external stimulation from the environment. The adult *Drosophila* visual system comprises the retina and optic lobe, which consists of the lamina, medulla, lobula, and lobula plate. Approximately 750 small eyes, ommatidia, are present in the retina, each containing eight photoreceptor neurons (R1–8). R1–6 are responsible for light-sensing and motion detection, while R7 expresses UV- and R8 blue- and green-sensitive opsins. Photoreceptors in *Drosophila* mainly release inhibitory neurotransmitter histamine to their postsynaptic neurons.

In the adult *Drosophila* visual system, upon continuous light condition (LL), photoreceptor neurons, especially R8, reorganize synaptic components at the Active Zone (AZ) through microtubule destabilization. The activity of both pre- and postsynaptic neurons are involved in synaptic component reorganization. However, how the visual system adapts to different environmental experiences at a circuitry scale is unknown. Moreover, while the presynaptic side is intensely studied during synaptic plasticity, we know rather less about the postsynaptic side in the adult *Drosophila* visual system. To

achieve a better understanding of the subsequent influence of activity-dependent synaptic plasticity caused by environmental stimulation to the circuitry in the *Drosophila* visual system, I applied a transcriptional reporter of intracellular calcium (TRIC) to monitor the activity of neurons postsynaptic to photoreceptors. The validation of postsynaptic physiological response showed that lamina monopolar neuron L2 tended to show more drastic activity response to environmental stimulation after activity-dependent synaptic plasticity caused by chronic light treatment in *Drosophila* visual system which was reversible if flies were returned to DD condition.

It attracted my interest in the underlying mechanism of response changes in the circuit to subsequent environmental stimulation following activity-dependent synaptic plasticity. Since histamine is the main and inhibitory neurotransmitter released by photoreceptors in *Drosophila* visual system, the expression level of histamine receptors (HRs) in L2 may account for the response change. I labeled HRs in postsynaptic L2 neurons and identified activity-dependent, transcriptional, reversible downregulation of HR in the medullar part of L2 neuron under LL condition. I found that HR decrease in L2 neurons was regulated by CaMKII and CREB-B. By contrast, histamine receptor downregulation does not occur in L1 upon prolonged light exposure, which is consistent with the result in TRIC assay that response changes happen only in L2, not in L1. The activity of R1–6 was proved necessary for this histamine receptor regulation in L2 through *ninaE* mutation experiment. I confirmed synaptic connections between R8 and L2 in the medulla and found that activating R8 was sufficient to downregulate the histamine receptor level in L2. The activation of L2 was also proved to stabilize the presynaptic components in presynaptic R8 photoreceptors.

Hereby, I report that in the adult *Drosophila* visual system, after constant light exposure-induced synaptic plasticity, inhibitory neurotransmitter histamine receptor transcriptional downregulation occurs in the postsynaptic lamina neuron L2, but not L1, depending on the photoreceptor and postsynaptic neuronal activity and involving CaMKII and CREB-B. The histamine receptor transcriptional downregulation results in a more intense postsynaptic L2 neuronal response to subsequent environmental stimulations, especially in the medulla. This research succeeded to visualize the circuit change process after synaptic plasticity according to long-term activation and find in vivo evidence of the circuitry plasticity. Findings in this research deepen the understanding of the consequence of activity-dependent synaptic plasticity to the circuit and provide new insights into the activity-dependent synaptic plasticity mechanism in the neural circuit.

1. Introduction

Environment, experience, and plasticity

Previous studies in rats reveal that animals raised in conditions with environmental enrichment (EE), given abundant interactive objects such as running wheels and toys and so on, can have better learning and memory performance, relatively larger brains, and more complicated neuronal circuits compared with those raised in boring housing condition with no social interaction (Alwis and Rajan, 2014; Hirase and Shinohara, 2014). It indicates that environment and experience can shape animals' neuronal circuits and affect brain development.

The ability that animals adapt to the environment through modifying the structure, connection, or molecular process in their brains is called 'plasticity'. Plasticity allows animals to develop mature brains, recover from injury, and respond to various stimuli and experiences from the environment. Plasticity occurring during adaption happens in different scales. On the cellular or morphological scale, neurogenesis, dendritic branching, synaptic density, and wiring pattern can be modified. On the subcellular scale, plasticity occurs at the sites where neurons make connections with each other known as synapses. Synapses allow one neuron (presynaptic neuron) to pass a chemical or electrical signal to another (postsynaptic neuron). For chemical synapses, presynaptic neurons will release chemical substances called neurotransmitters which are contained in synaptic vesicles. Postsynaptic neurons can receive neurotransmitters via corresponding neurotransmitter receptors and trigger responses (Fig. 1A). Upon different environmental experiences, presynaptic component expression, neurotransmitter level, receptor expression can be adjusted, in result changing the pattern and information flow among synaptic-forming neurons (Alwis and Rajan, 2014) (Fig. 1B).

Activity-dependent synaptic plasticity and related pathways

Activity-dependent synaptic plasticity, components of activity-dependent neuroplasticity in the nervous system, is believed to be crucial for responses to the environment and adaptive behaviors. Previous studies have demonstrated the complex and delicate mechanisms of synaptic modulation including reorganization of synaptic components (Packard et al., 2002; Packard et al., 2003; Okamoto et al., 2004; Sugie et al., 2015), regulation of neurotransmitter release, and many other processes to adjust the connection strength or information flow according to environmental stimulation.

In the mammalian central nervous system, activity-dependent synaptic plasticity is well-studied in learning and memory (Neves et al., 2008), mostly regarding to long-term potentiation (LTP) or long-term depression (LTD) (Song and L.Huganir, 2002; Ho et al., 2011). Numerous pre- and postsynaptic mechanisms of plasticity have been found at excitatory chemical synapses in the rodent hippocampus (Ho et al., 2011; Buonarati et al., 2019). At the presynaptic terminal, the trafficking, recycling, and release process of synaptic vesicles with neurotransmitters at the Active Zone (AZ) can be regulated by an activity-dependent pathway which is triggered by calcium influx (Ho et al., 2011).

However, at the postsynaptic terminal, the calcium influx, through activated NMDA receptors (NMDARs), is also needed for the induction of LTP. Postsynaptic regulation of AMPA glutamate receptors (AMPARs), involving the trafficking and recycling of the receptors, is crucial for plasticity (Raymond et al., 1993; Song and L.Huganir, 2002; Ho et al., 2011). Calmodulin kinase II (CaMKII) can phosphorylate AMPARs due to calcium influx, and in consequence modulate the channel activity of AMPARs, lead AMPARs to the site of PSD-95, and finally induce LTP (Lisman et al., 2002; Okamoto et al., 2004; Lee et al., 2009). The exocytosis and endocytosis of AMPARs account for the AMPAR level at the synapse due to LTP or LTD (Buonarati et al., 2019). These findings imply that the postsynaptic modification during activity-dependent synaptic plasticity is mainly through regulating the localization and recycling of the postsynaptic receptors, and CaMKII plays a big role in the processes.

In recent years, some studies show that transcriptional regulation in the postsynaptic terminal also occurs during activity-dependent synaptic plasticity (Yin et al., 2018; Mazaud et al., 2019). Transcriptional factor cAMP response element-binding protein (CREB) functions after calcium influx to manipulate the expression of downstream activity-regulated genes (Carlezon et al., 2005). CaMKII is known to inhibit CREB by phosphorylating serine 142 in CREB which leads to dissociation of the CREB dimer (Matthews et al., 1994; Wu and McMurray, 2001). In developing *Drosophila* visual circuit, lipophorin receptors LpR1 and LpR2 in the postsynaptic ventral lateral neurons (LNvs) can be upregulated during constant light exposure (LL) (Yin et al., 2018). *CrebB* is also one of the activity-dependent genes regulated by the light exposure in the study (Yuan et al., 2011; Yin et al., 2018).

Synaptic plasticity and circuit change

Many studies have been conducted to look at activity-dependent synaptic plasticity occurring between one neuron and another. It still requires more studies on how synaptic

plasticity affects the circuit change. When a circuit receives input activity, direct synaptic changes occur and have subsequent consequences in the circuit. One type of presynaptic neuron usually has connections with various types of postsynaptic neurons. It remains a big question whether synaptic plasticity happens among all the connections, or only some specific subsets of postsynaptic neurons change the connections with the presynaptic neuron and modify their activity responses according to input activity. (Fig. 2)

The purpose of my research is to visualize the circuit change process after synaptic plasticity according to long-term activation and find in vivo evidence of the circuitry plasticity upon long-term environment stimulation.
After a circuit receives long-term activation, three questions remain to be answered:
-Where, or in which types of postsynaptic neurons will the plasticity occur?
-How is the phenomenon, or what kind of plasticity changes will there be in the circuit?
-Why does the change occur? If only some specific subsets of neurons show plasticity, what is the mechanism underlying it?

Drosophila, and commonly used genetic tools

To achieve the purpose of my research, I conducted experiments in model animal *Drosophila Melanogaster*, which is also generally called 'fruit fly', because for the activity-dependent plasticity research at the synaptic level, plenty of genetic tools and experimental methods have already been developed in *Drosophila*.
Drosophila has been used as the model organism to study various biological mechanisms for long years (Stephenson and Metcalfe, 2013). Many findings of great significance in advanced organisms such as rats, mice, and humans are based on tools or results derived from *Drosophila*. Easy rearing and rapid reproduction, short life span, and small-sized bodies make *Drosophila* an ideal model animal for large-scale behavior analysis and molecular screening. Numerous mutant line stocks and powerful genetic tools make it easy for researchers to cross or generate expected genotypes. Experiment design can be largely flexible with various tools and crossing strategies. (Bai and Suzuki, 2020)
The commonly used genetic tools applied in my research are the Gal4-UAS system, $Gal80^{ts}$ (temperature-sensitive version of the GAL80 protein), TRPA1 (Transient receptor potential cation channel A1), and FRT-STOP-FRT (FRT-flanked transcriptional stop cassette).

1) Gal4-UAS system

The Gal4-UAS system is used to study gene expression and function in the fruit fly. It is

composed of two parts: the transcription activator protein Gal4 derived from yeast, and the upstream activation sequence (UAS), which is an enhancer. Gal4 protein can specifically bind to UAS to trigger the gene transcription downstream (Fig. 3A). For example, for the neuroscience research in the fruit fly, the Gal4-UAS system allows specific gene expression in target neuron subsets just by crossing Gal4 lines (expressing Gal4 in some subsets of neurons) and UAS lines (specific genes following UAS).

2) Gal80^{ts} (temperature-sensitive version of the GAL80 protein)

The Gal4-UAS system enables concise regional control of gene expression but lacks temporal control to express the specific genes in specific neurons in defined periods. The GAL80 protein, which normally functions as a repressor of Gal4, has a temperature-sensitive version named Gal80^{ts}. Gal80^{ts} can act as an 'ON/OFF switch' for Gal4-UAS system under a temperature-sensitive manner through conformation change. It allows Gal4-UAS to function at a temperature over 29 °C and represses Gal4 at 18 °C (Fig. 3B). (McGuire et al., 2003)

3) TRPA1 (Transient receptor potential cation channel A1)

Transient receptor potential channels are responsible for thermosensation. TRPA (transient receptor cation potential A) channels are commonly applied in *Drosophila* and TRPA1 enables artificial activation of tissues at a temperature over 29 °C (Luo et al., 2017). In neuroscience research in *Drosophila*, flies are placed in an incubator of which the temperature is set at over 29 °C to trigger calcium signals via TRPA1 expressed in target neurons, and therefore specific neurons can be activated.

4) FRT-STOP-FRT (FRT-flanked transcriptional stop cassette)

FLP-FRT recombination is a useful genetic tool that allows site-directed recombination. It is composed of an FRT-flanked cassette which contains target sequences between two flippase recognition target (FRT) sites, and FLP (flippase), a kind of recombinase derived from yeast. Using FLP can flip out FRT-flanked sequences which have been inserted in advance to the DNA. (Chen et al., 2014; Li et al., 2020)

FRT-STOP-FRT cassette contains transcriptional stop sequence flanked by two FRT sites. With cell-specific FLP construct, or neuron-specific Gal4 lines and UAS-FLP, the FRT-STOP-FRT cassette can be removed in specific subsets of neurons, which in result allows expression of target genes in the downstream (Fig. 3C). This method is used to express genes in a neuron type-specific and concise manner.

Synaptic plasticity in *Drosophila* visual system

The *Drosophila* visual system, either in the developing or adult stage, is a powerful model to study the activity-dependent synaptic plasticity invoked by external stimulation from the environment (Yuan et al., 2011; Sugie et al., 2018). The adult *Drosophila* visual system comprises the retina and optic lobe. The optic lobe consists of lamina, medulla, lobula and lobula plate. There are estimated 750 small eyes called ommatidia in the retina and each ommatidium has eight types of photoreceptor neurons (R1 to R8). R1 to R6 are responsible for the light-sensing and motion detection, while R7 expresses UV-sensitive opsins and R8 expresses blue- and green-sensitive opsins (Alejevski et al., 2019). R8 is believed to account for the color-sensing function. R1–6 photoreceptors project to the lamina, while R7 and R8 photoreceptors extend to the M6 and M3 layer of medulla respectively (Fig. 4A). Photoreceptors in *Drosophila* mainly release inhibitory neurotransmitter histamine to their postsynaptic neurons (Alejevski et al., 2019). Histamine receptors in the mammalian brain are mostly G protein-coupled receptors. In *Drosophila*, however, histamine receptors in neurons postsynaptic to photoreceptors are histamine-gated chloride channels. Receiving histamine can cause hyperpolarization in the neuron, decrease the activity (Dau et al., 2016).

In previous research regarding adult *Drosophila* visual system, upon continuous light condition (LL), photoreceptor neurons, especially R8, reorganize synaptic components at the Active Zone (AZ) such as Brunchpilot (Brp), DSyd-1, Cacophony, and so on through microtubule destabilization (Fig. 4B) (Sugie et al., 2015). The activity of both pre- and postsynaptic neurons are involved in synaptic component reorganization. This kind of synaptic component reorganization can be reversed when flies are returned to continuous dark condition (DD) or normal light-dark condition (LD) for 72 hours. Wingless (Wg) signaling pathway is revealed to participate in the process of microtubule destabilization and regulate synaptic component reorganization which happens at the AZ (Sugie et al., 2015; Kawamura et al., 2021). However, the circuitry response to subsequent environmental stimulation after activity-dependent synaptic plasticity remains poorly understood. Moreover, while the presynaptic side is intensely studied during synaptic plasticity, we know significantly less about the postsynaptic side in the adult *Drosophila* visual system.

To achieve a better understanding of the subsequent influence of activity-dependent synaptic plasticity caused by environmental stimulation to the circuit in the *Drosophila* visual system, I applied a transcriptional reporter of intracellular calcium (TRIC) to the neurons postsynaptic to photoreceptors. TRIC can express GFP in the neuron where

calcium influx (representing for neuronal activity) occurs via binary system (Fig. 5) (Gao et al., 2015). The validation of physiological response via TRIC showed that only some subsets of postsynaptic neurons such as lamina monopolar neuron L2 tended to show more drastic activity response to environmental stimulation after activity-dependent synaptic plasticity caused by chronic light treatment in *Drosophila* visual system. Even though lamina monopolar neuron L1 also has connections with photoreceptors, there was no enhanced activity response in L1 according to the pan-neuronal TRIC experiment.
It attracted my interest in the underlying mechanism of response changes in the circuit to subsequent environmental stimulation following activity-dependent synaptic plasticity. The presynaptic remodeling with a decrease in synapse number may be one of the reasons. However, the slight decrease in synapse number cannot solely explain the robust circuitry response change in postsynaptic neurons caused by activity-dependent synaptic plasticity. Thus, I set out to search for other molecular mechanisms underlying this phenomenon. Since histamine is the main and inhibitory neurotransmitter released by photoreceptors in *Drosophila* visual system, and histamine receptors (HRs) in Drosophila are histamine-gated chloride channels, the expression level of HRs in L2 may be the answer to the question. I labeled HRs in postsynaptic L2 neurons and identified downregulation of HR in the medullar part of L2 neuron under LL condition, and the HR expression decrease was reversible. I also found that HR decrease in L2 neurons was activity-dependent and transcriptional, regulated by CaMKII and CREB-B. By contrast, histamine receptor downregulation does not occur in L1 upon prolonged light exposure, which is consistent with the result in TRIC assay that response changes happen only in L2, not in L1. The activity of R1–6 was proved necessary for this histamine receptor regulation in L2 through *ninaE* mutation experiment which blocked the signals from R1–6. I checked the synaptic connection between L2 and photoreceptors using the trans-Tango method (Talay et al., 2017) and GFP Reconstitution Across Synaptic Partners (GRASP) technique (Feinberg et al., 2008; Macpherson et al., 2015) and confirmed the synaptic connection between R8 and L2 in the medulla. I also found that activating R8 was sufficient to downregulate the histamine receptor level in L2.

Hereby, I report that in the adult *Drosophila* visual system, after constant light exposure-induced synaptic plasticity, inhibitory neurotransmitter histamine receptor transcriptional downregulation occurs in the postsynaptic lamina neuron L2, but not L1, depending on the photoreceptor and postsynaptic neuronal activity and involving CaMKII and CREB-B. The histamine receptor transcriptional downregulation results in a more intense postsynaptic L2 neuronal response to subsequent environmental stimulations, especially

in the medulla. This research succeeded to visualize the circuit change process after synaptic plasticity according to long-term activation and find in vivo evidence of the circuitry plasticity upon long-term environment stimulation. Findings in this research deepen the understanding of the consequence of activity-dependent synaptic plasticity to the circuit and provide new insights into the activity-dependent synaptic plasticity mechanism in the neural circuit.

2. Material and Methods

1. Fly food

The ingredients of fly food and amounts were as follows:

Ingredients	**Amounts**
Water	1 L
Soybean	15 g
Agar	10 g
Cornmeal	100 g
Yeast	30 g
Malt	30 g
Glucose	50 g

The mixture was heated at 90 °C for 1 hour. After that, 5 mL ethanol, 2 mL propionic acid, and 2.5 g Nipagin were added, and then the mixture was dispensed into vials and bottles.

2. Fly husbandry

Flies were raised in vials containing a 2 cm cornmeal, yeast-based food layer. The vials were placed in plastic tray vial containers and stored in different incubators depending on purposes. For expansion, fly lines were kept in 25 °C and 60% humidity incubators with the 12-hour light, 12-hour dark cycle, also known as LD. The fly stocks were kept at 18 °C dark room. The temperature and humidity of the incubators were kept at a constant value.

3. Light stimulation

The environment change was stimulated as light conditions change. The normal flies were reared in 12-hour light, 12-hour dark cycle. For generating the constant condition, I reared the flies in,

1) 24-hour dark (continuous dark/DD) in 25 °C or 18 °C incubator

2) 24-hour light (continuous light/LL) in 25 °C or 18 °C incubator [light intensity 4000 lux]

4. TRIC assay and Gal80^{ts}

Flies carrying TRIC components (LexAop2-mCD8GFP,UAS-mCD8RFP;nSyb-nlsLexADBDo;UAS-p65ADCaM) were crossed with Gal4 lines. On day 3, adult flies were transferred, and vials were covered by foil paper, placed at 25 °C for 8 days until eclosion. On day 10, flies were collected and divided into several groups according to experimental requirements.

GAL80^{ts}, a temperature-sensitive version of the GAL80 protein, functions as a repressor of GAL4, and temperature-sensitive regulation of GAL4 activity can be realized by conformation change of Gal80^{ts} in different temperatures. Gal80^{ts} is used to restrict reporter availability of the TRIC system until eclosion (McGuire et al., 2003). Flies carrying TRIC components (LexAop2-mCD8GFP,UAS-mCD8RFP;nSyb-nlsLexADBDo;UAS-p65ADCaM) were crossed with Gal4 lines which contained Gal80^{ts} (w;Gal80^{ts}/CyO;X-Gal4/TM6B). On day 3, adult flies were transferred, and vials were covered by foil paper, placed at 25 °C for 2 days. On day 5, the vials were moved to an 18 °C incubator. On day 15, flies were collected and divided into several groups according to experimental requirements (Fig. 6).

5. Quantification of synapse numbers using Brp protein

Brp is the presynaptic component that localizes to T-bars, platforms for vesicle assembly in Active Zone, representing for the synapse. The Brp protein is marked by GFP following the removal of an FRT-flanked transcriptional stop cassette FRT-STOP-FRT, constructed in the bacterial artificial chromosome (BAC). The FRT-STOP-FRT cassette can be removed specifically in photoreceptors using sensFLP to allow the specific expression of GFP-marked Brp protein in photoreceptors. The number of Brp-GFP BAC puncta in axons was counted in a blinded manner. (Chen et al., 2014; Sugie et al., 2015)

6. OLLAS staining assay

Flies carrying Ort(FRT.SA.Stop)OLLAS;UAS-FLP (Chen et al., 2014) were crossed with L2-specific Gal4, along with UAS-myr-RFP or not according to experimental

requirements. In some experiments, flies with recombined 27G05FLP (pan-lamina FLP) and Ort(FRT.SA.Stop)OLLAS on the second chromosome and other components on the third chromosome were crossed with UAS-myr-RFP;L2-specific Gal4. Anti-OLLAS antibody (Funakoshi Anti-OLLAS 0.5 ml NBP1-06713) was used at the concentration of 1:200 and incubated overnight at 4 °C with shaking. During the second staining, Alexa FluorTM 488 goat anti-rat IgG(H+L) (Life Technologies) were added (1:400) and incubated at room temperature for 2 hours with shaking. After confocal imaging, measurements were done on the IMARIS2 measurement pro track (Carl Zeiss).

7. Fly brain dissection and Immunohistochemistry

Fly brains were dissected in 0.1% PBT which was composed of phosphate buffer saline (PBS) and 0.1% Triton X-100 and fixed in fixation solution (4% paraformaldehyde in 0.1% PBT) for 1 hour. After fixation, brains were washed with 0.1% PBT three times and incubated in PBT for 1 hour at room temperature. Brains were then incubated in primary antibody overnight at 4 °C. Following first staining with primary antibody, brains were washed with PBT three times and then incubated in secondary antibody for 1 or 2 hours at room temperature. Following second staining with secondary antibody, brains were washed with PBS two times and mounted in Vectashield (Vector Lab), ready for confocal imaging.

If the experiment does not require immunostaining, after fixation, brains were washed with 0.1% PBT three times and incubated in PBT for 1 hour at room temperature. After that, brains were directly washed with PBS twice and mounted in Vectashield, ready for confocal imaging.

8. Antibody

Antibodies used in this research were as follows:

Antibody	**Maker**	**Concentration**
Primary antibody		
mAb24B10	DSHB	1:50
Anti-OLLAS	Funakoshi	1:200
Second antibody		

Goat anti-rat IgG(H+L) Alexa Flour 488 conjugate	Life Technologies	1:400

9. Confocal imaging and image quantification

Dissection was carried out using Leica light microscope. All confocal images were captured using Nikon Eclipse Ni microscope and Nikon C2plus Confocal microscope. Scanning was done with a step size of 0.5-1 μm in a sample cross-section size of 15-30 μm. The 3D images are acquired using NIS-Element AR (Nikon). After confocal imaging, quantifications were done using the IMARIS2 measurement pro track (Carl Zeiss).
For histamine receptor intensity quantification, the surfaces of L2 neurons were defined by IMARIS2 via L2-specific RFP or mcherry fluorescence signal, and the average staining green fluorescence signal intensity in L2 was calculated in a weighted average manner based on the volumes of different defined regions. The average intensity of the randomly selected area in lobula was subtracted from the original data to eliminate the staining background.

10. RNA extraction and reverse transcription

RNA was extracted from 10 flies for each experiment condition and the process followed the protocol of Promega ReliaPrepTM RNA Tissue Miniprep System kit (Promega, Z6110). Extracted RNA was stored in a -70 °C refrigerator. ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO, FSQ-301) was used to synthesize cDNA from extracted RNA for real-time PCR.

11. qPCR

qPCR reaction mixtures were prepared using THUNDERBIRD® SYBR® qPCR Mix (TOYOBO, QPS-201) and the process followed the protocol. qPCR was done via Real-time PCR equipment TP970 (Takara) and analyzed by Thermal Cycler Dice® Real Time System (Takara).

qPCR reaction mixture setup

Reagent	Volume (μL)
THUNDERBIRD® SYBR® qPCR Mix	20
Forward primer	1.2
Reverse primer	1.2
Reverse transcription DNA mixture	4
Water	13.6

Primers were designed to target OLLAS-GS-Ort, which indicated the mRNA level of OLLAS-tagged histamine receptor protein ORT. Three independent biological replicates and several technical replicates for each biological replicates were measured for LL or DD conditions. Housekeeping gene *Rpl32* was used as a reference, and primers were referred from FlyPrimerBank.

qPCR primers

OLLAS-GS-Ort	
CCTGATGGGCAAGGGTGG	Forward primer
CTTCGGCGGTCTCATCTTGT	Reverse primer
Housekeeping gene Rpl32	
CGGATCGATATGCTAAGCTGT	Forward primer
CGACGCACTCTGTTGTCG	Reverse primer

qPCR program settings

Step	Temperature (°C)	Time length (second)	
Pre-denaturation	95	30	
Denaturation	95	5	45 cycles
Annealing	55	10	
Extension	72	30	
Dissociation	95	15	
	60	30	
	95	15	

The calculation of threshold cycle (Ct) of qPCR followed 2nd Derivative Maximum method.

The relative quality of LL compared to DD (defined as 1) was calculated as follows:

	OLLAS-GS-Ort (Ct average)	Rpl32 (Ct average)	dCt	ddCt	Relative quality
DD	A	C	E (=A-C)	0	1
LL	B	D	F (=B-D)	G (=F-E)	2^(-G)

12. Statistical Analysis

Statistical analysis between two groups was performed using a two-tailed Student's t-test. Statistical analysis among more than two groups was performed using non-parametric Kruskall-Wallis H test, followed by post hoc pairwise Mann-Whitney U tests. All *p* values were corrected according to the Holm-Bonferroni method to control for the false discovery rate within multiple comparisons.
Statistical analysis was performed using the website:
https://www.statskingdom.com/kruskal-wallis-calculator.html
Significance is shown by asterisks in figures as follows: *p<0.05, **p<0.01, ***p<0.001.

13. Fly stocks

Fly stocks were as follows:

Genotypes	**Source**	**Note**
ninaE^{17}	Bloomington BDSC_5701	*ninaE* mutant
LexAop2-mCD8GFP,UAS-mCD8RFP;nSyb-nlsLexADBDo;UAS-p65ADCaM	Bloomington BDSC_61679	TRIC
GMR16H03Gal4	Bloomington BDSC_48744	L2-specific Gal4
nSybGal4	Bloomington BDSC_51635	Pan-neuronal Gal4
Rh6Gal4	Bloomington BDSC_7464	R8-specific Gal4
tubP-Gal80^{ts}	Bloomington	Gal80^{ts}

	BDSC_7108	
Ort(FRT.SA.Stop)OLLAS	Bloomington BDSC_55761	OLLAS-tagged histamine receptor (ORT)
Ort(FRT.SA.Stop)OLLAS;UAS-FLP	Bloomington BDSC_55762	
R27G05-FLPG5.PEST	Bloomington BDSC_55765	Pan-lamina FLP
R48A08-p65.AD(attP40); MKRS/TM6B, Tb[1]	Bloomington BDSC_70707	L1-AD
R66A01-GAL4.DBD(attP2)	Bloomington BDSC_68999	L1-DBD
UAS-nSyb-spGFP1-10,lexAop-CD4-spGFP11	Bloomington BDSC_64314	GRASP
UAS-myrGFP,QUAS-mtdTomato-3xHA;trans-Tango	Bloomington BSDC_77124	trans-Tango
UAS-TrpA1	Bloomington BSDC_26264	TRPA1
CaMKII-RNAi	Bloomington BSDC_29401	
UAS-CrebB	Bloomington BSDC_7220	
CrebB-RNAi	Bloomington BSDC_63681	
UAS-ort (2^{nd})	Chi-Hon Lee	
27G05-FLPG.PEST(attP5)	Matthew Y. Pecot	Pan-lamina FLP inserted in attP5 site

14. Genotypes

The genotypes used in figures were as follows:

Figure 7	LexAop2-mCD8GFP,UAS-mCD8RFP;nSyb-nlsLexADBDo/Gal80^{ts};UAS-p65ADCaM/nSybGal4

Figure 8	LexAop2-mCD8GFP,UAS-mCD8RFP;nSyb-nlsLexADBDo/Gal80^{ts};UAS-p65ADCaM/GMR16H03Gal4
Figure 9	LexAop2-mCD8GFP,UAS-mCD8RFP;nSyb-nlsLexADBDo/Gal80^{ts};UAS-p65ADCaM/GMR16H03Gal4
Figure 10	LexAop2-mCD8GFP,UAS-mCD8RFP;nSyb-nlsLexADBDo/Gal80^{ts};UAS-p65ADCaM/GMR16H03Gal4
Figure 12	UAS-myr-RFP/Ort(FRT.SA.Stop)OLLAS;GMR16H03Gal4/UAS-FLP
	Ort(FRT.SA.Stop)OLLAS/+;GMR16H03Gal4/UAS-FLP
Figure 13	UAS-myr-RFP/Ort-FSF-OLLAS; GMR16H03Gal4/UAS-FLP
Figure 14A-C	UAS-myr-RFP/Ort-FSF-OLLAS; GMR16H03Gal4/UAS-FLP
Figure 15	Ort(FRT.SA.Stop)OLLAS,R27G05FLPG5.PEST(attP5)/LexAop-mcherry,GMR16H03-LexA; ninaE^{17}
Figure 16A	UAS-nSybspGFP1-10,LexAopCD4GFP/GMR16H03-LexA,LexAopmcherry;GMR-Gal4
Figure 16B	SensFLP/UAS-myr-GFP,QUAS-mtdTomato-3xHA;GMR-fsf-Gal4/trans-Tango
Figure 16C	UAS-nSybspGFP1-10,LexAopCD4GFP/GMR16H03-LexA,LexAopmcherry;Rh6-Gal4
Figure 17	Ort(FRT.SA.Stop)OLLAS,R27G05FLPG5.PEST(attP5)/LexAop-mcherry,GMR16H03-LexA; Rh6-Gal4, UAS-TrpA1
Figure 18	Ort(FRT.SA.Stop)OLLAS/UAS-TrpA1;GMR16H03-Gal4/UAS-FLP
Figure 19A	Ort(FRT.SA.Stop)OLLAS,R27G05FLPG5.PEST(attP5)/UAS-myr-RFP; GMR16H03-Gal4
	Ort(FRT.SA.Stop)OLLAS,R27G05FLPG5.PEST(attP5)/UAS-myr-RFP; GMR16H03-Gal4/CaMKII-RNAi
	Ort(FRT.SA.Stop)OLLAS,R27G05FLPG5.PEST(attP5)/UAS-myr-RFP;GMR16H03-Gal4/UAS-CrebB
Figure 19B	Ort(FRT.SA.Stop)OLLAS;GMR16H03-Gal4/UAS-FLP
	Ort(FRT.SA.Stop)OLLAS/UAS-CrebB-RNAi;GMR16H03-Gal4/UAS-FLP
Figure 20	13XLexAop2-mCD8::GFP,10XUAS-IVS-mCD8::RFP/w;nSyb-MKII::nlsLexADBDo/+;UAS-p65AD::CaM,tubP-GAL80^{ts}/ GMR16H03-GAL4

	13XLexAop2-mCD8::GFP,10XUAS-IVS-mCD8::RFP;nSyb-MKII::nlsLexADBDo/UAS-ort;UAS-p65AD::CaM,tubP-GAL80^{ts}/ GMR16H03-GAL4
Figure 21A	UAS-FLP;L1-AD/Ort-FSF-OLLAS; L1-DBD/UAS-myr-RFP
Figure 21B	L1-AD/Ort-FSF-OLLAS; L1-DBD/UAS-FLP
Figure 22	SensFLP/w;+/+;BAC(Brp-FSF-GFP)/+
	SensFLP/w;GMR16H03-Gal4/+;BAC(Brp-FSF-GFP)/UAS-TrpA1

3. Results

1. Specific post-synaptic neurons in the medulla show different activity responses towards photoreceptor activation after various light treatments

In the previous study, it showed that the number of synapses in photoreceptor R8 of *Drosophila* reduced after a 3-day LL condition. The synapse number decrease mainly happened in the M2 layer, the second layer of medulla. The result indicated that the presynaptic components can be adjusted to adapt to different light environments. However, it remains unknown what plasticity changes will happen in postsynaptic neurons, and whether synaptic plasticity caused by light conditions affects the circuitry responses towards subsequent photoreceptor activation or not. Thus, after 6-day LL or DD treatment, I activated photoreceptors on day 7 and examine the activity response of the visual system at that time.

To measure the activity response of the related circuit, I used the Transcriptional Reporter of Intracellular Ca^{2+} (TRIC) method, allowing GFP expression in neurons where calcium influx occurs with a binary system (Gao et al., 2015) (Fig. 5). When calcium influx occurs, calmodulin interacts with its target peptides and allows subsequent expression of reporter genes such as GFP. To measure the calcium influx only on day 7 and avoid saturation of TRIC signals during the development stage, I restricted TRIC reporter availability until day 7 via the temperature-sensitive GAL80 repressor ($GAL80^{ts}$). $GAL80^{ts}$ functions as an on-off switch of the GAL4-UAS system since it functions as a repressor of GAL4 at 18 °C and will not repress GAL4 over 29 °C (McGuire et al., 2003). Flies are raised according to a specific protocol to avoid heavy background during the development stage and treated by continuous light (LL) or continuous dark conditions (DD) for 6 days under 18 °C after eclosion. After that, I activated photoreceptors through 1-day continuous light condition under 32 °C on day 7, to test the circuitry activity response towards this 1-day photoreceptor activation (Fig. 6). I expressed the TRIC system pan-neuronally and found that some neurons with oval axon terminals in the medulla, mostly likely the lamina monopolar neuron L2, showed significantly higher levels of TRIC GFP signals towards 1-day light activation after 6-day LL treatment than the ones treated with 6-day DD (Fig. 7). Upon 6-day LL treatment and 1-day activation, an average of 12.5 neurons with oval axon terminals in the medulla exhibited a TRIC signal within 15 μm range of depth, while few TRIC-positive neurons could be observed with oval structure in the medulla

with 6-day DD and 1-day activation. However, even though lamina monopolar neuron L1 is also postsynaptic to photoreceptors, activity response did not increase in L1-like neurons upon 1-day activation after 6-day LL, since there was no obvious TRIC signal change in M1 or M5 layer according to pan-neuronal TRIC experiment. It indicates that only some specific subsets of post-synaptic neurons in medulla, not all of them, display enhanced activity responses towards subsequent photoreceptor activation after long-term light treatments.

2. Lamina monopolar neuron L2 responds more drastically to photoreceptor activation after prolonged light exposure

To further understand the change in activity response, I concentrated efforts on L2 neurons and expressed the TRIC system specifically in L2 (GMR16H03Gal4). Our results showed significantly more L2 neurons in flies treated with 6-day LL condition (in average 7.8 neurons within 15 μm range of depth) exhibiting bright TRIC signals after activation, than the ones treated with 6-day DD condition (in average 1.6 neurons within 15 μm range of depth). Moreover, these signals were proven not to derive from incomplete $GAL80^{ts}$ repression (Fig. 8), since I observed significantly less TRIC positive signal without 1-day activation. Flies treated with 6-day LD condition showed no significant difference in the number of TRIC-positive L2 neurons after 1-day activation compared with those treated with 6-day DD. The result confirms that DD condition has no significant effect on the activity response of L2 compared with the normal LD condition (Fig 9).
I put the flies, previously treated by 6-day LL, back to the DD environment for 6 more days and observed that the TRIC GFP signals disappeared (Fig. 10). These results indicate that the changes caused by various light conditions affect the L2 neuronal response towards subsequent photoreceptor activation, and this response type difference caused by the continuous light treatment is reversible.

3. The histamine receptor level in the medullar part of the L2 neurons is regulated by light conditions

Among all the neurons, it seemed that only several specific subsets of neurons like L2 showed increased activity response towards photoreceptor activation after prolonged light exposure. Other lamina monopolar neurons such as L1 did not show increased TRIC-

positive signal according to the experiment with a pan-neuronal expression of TRIC. It attracted my interest in the underlying mechanism of response change. There must be something working differently in L1 and L2 under the LL condition. It remains unclear what kind of plasticity occurring in postsynaptic neurons causes the activity response change. The presynaptic remodeling-based reduction in the synapse number between the L2 and the photoreceptors may account for the L2 activity response change (Sugie et al., 2015; Kerwin et al., 2018) because the decreased number of synapses could reduce the inhibitory neurotransmitter release from photoreceptors. However, since the presynaptic remodeling with a slight reduction in the number of synapses cannot solely explain the robust circuitry response change in postsynaptic neurons caused by activity-dependent synaptic plasticity, I put up another possible reason that the L2 activity change derived from the histamine receptor reduction in the postsynaptic neurons besides synapse decrease.

Since histamine is an inhibitory neurotransmitter released from *Drosophila* photoreceptors (Dau et al., 2016) and histamine receptors (HRs) in Drosophila are histamine-gated chloride channels, the reduced number of HRs might trigger more calcium influx in L2. I used OLLAS (OmpF Linker and mouse Langerin fusion Sequence) as a tag linked with ORT protein (HR protein in postsynaptic neurons of photoreceptors) to label the HR (Chen et al., 2014). To achieve neuron-type-specific expression of tagged ORT proteins, FRT-flanked transcriptional and translational stop cassette with a splice acceptor (SA) was inserted in the upstream of the OLLAS-tagged *Ort* gene. Only when combined with a neuron-type-specific FLP recombinase can the stop cassette be removed, allowing the expression of OLLAS-tagged ORT protein (Chen et al., 2014) (Fig. 11). In my research, through expressing FLP specifically in L2 neurons, tagged HRs were observed only in L2 through OLLAS-staining. The result showed that the intensity of OLLAS signals in the medullar part of L2 was significantly decreased under the 6-day LL condition compared to 6-day DD. However, there was no significant decrease of L2-specific tagged HR after 1-day or 3-day LL, which indicated that HR downregulation happened at least after 3 days (Fig. 12A).

To confirm whether this kind of HR downregulation is transcriptional or not, reverse transcriptional PCR and real-time PCR were applied to measure the mRNA level of L2-specific tagged HRs. RNAs were extracted from the whole body, and then cDNAs were made through reverse transcriptional PCR. Primers designed to target a segment of tagged HR (involving sequences of OLLAS, GS link, and *Ort*) were used to conduct the real-time qPCR. While the transcription of tagged HR in other tissues was prevented by FRT-STOP-FRT cassette, only the tagged HR of which FRT-STOP-FRT had been flipped out

by L2-specific FLP could be successfully transcribed (Fig. 11). Thus, I could measure the mRNA level of tagged HR specifically in L2. Results showed that the transcription level of L2-specific tagged HR significantly decreased after 6-day LL compared with DD. The mRNA level of L2-specific tagged ORT protein after 6-day LL treatment was only 22.8 percent of the one after 6-day DD treatment (Fig. 12B). The result shows that this kind of histamine receptor downregulation is transcriptional. However, after 3-day or 1-day LL, mRNA level of L2-specific OLLAS-tagged HR had no significant difference compared with 3-day or 1-day DD respectively. It indicates that transcriptional regulation of OLLAS-tagged HRs starts at least no earlier than 3-day. Prolonged light exposure lasting longer than 3-day should be necessary for the regulation of HRs in L2. Flies treated with 6-day LD condition showed no significant difference in OLLAS staining intensity compared with those treated with 6-day DD. The result confirms that the DD condition has no significant effect on the HR expression level in L2 compared with the normal LD condition (Fig 13). Moreover, the percentage of OLLAS-positive L2 neurons within the 15 μm range of depth was also decreased under the 6-day LL condition (74%) compared to 6-day DD (63%) (Fig. 14A). OLLAS staining GFP intensity significantly decreased from 522.96 (6-day DD) to 298.82 (6-day LL) of L2 neurons in the lamina, and significantly decreased from 261.56 (6-day DD) to 148.42 (6-day LL) of L2 neurons in the medulla (Fig. 14B). This result demonstrates that the HR level is downregulated both in the laminar and medullar parts of L2 neurons after prolonged light exposure. The decrease of HRs in the medullar part could indicate the overall HR downregulation in the L2 neurons. Moreover, this kind of downregulation was also confirmed to be reversible. The decreased HR expression level in L2 because of 6-day LL could be recovered if flies were put back to the DD environment for 6 more days (Fig. 14C).

4. R1–6 activities are necessary for the histamine receptor loss in the medullar part of L2 under continuous light conditions

Among 8 types of photoreceptors, R1–6 photoreceptors are responsible for light-sensing and maintain connections with L2 in the lamina. R1–6 photoreceptors are considered as the main input sources for L2 neurons. There is a high possibility that the activity of R1–6 may account for the HR downregulation in L2. Rhodopsin is the protein in photoreceptor that detect light and vision. In R1–6 photoreceptors, the rhodopsin type is Rh1 (ninaE). For the *ninaE* mutant, in which signals from R1–6 were blocked (Chou et al., 1996), even though HR expression level in the medullar part of L2 in flies treated with

6-day LL was slightly decreased 12.8% on average compared to those treated with 6-day DD, the difference was not significant. The results indicate that R1–6 activities are necessary for the histamine receptor loss in the medullar part of L2 under continuous light conditions. The slight decrease in HR level after LL treatment despite *ninaE* mutant implies other factors regulating the histamine receptor level (Fig. 15).

5. R8 photoreceptor has connections with L2 in medulla

According to previous studies, after LL treatment, the synapse number of R8 is decreased mainly in M2 layer (Sugie et al., 2015). It remains to be checked whether R8 photoreceptors have connections with L2 neurons in M2 layer or not. Using the activity-dependent GFP Reconstitution Across Synaptic Partners (GRASP) method to detect the active synaptic connection between two specific neurons (Feinberg et al., 2008; Macpherson et al., 2015), I found that photoreceptors establish connections with L2 in the medulla (Fig. 16A). Since only R7 and R8 photoreceptor neurons extend their axons to the medulla, it indicated the possibility that L2 neurons have connections with R7 or R8 in the medulla. I used the trans-Tango approach (Talay et al., 2017) to search for the second-order neurons of R8 and found the L2-like neurons with oval axon terminals (Fig. 16B). I further checked the connections between R8 and L2 via the activity-dependent GRASP technique and found that R8 photoreceptors have synaptic connections with L2 in the medulla (Fig. 16C).

6. Activating photoreceptor R8 is sufficient to induce the histamine receptor downregulation in the medullar part of L2 under LL condition

R8 photoreceptors are confirmed to have connections with L2 neurons in the medulla. To determine whether the activity of R8 can affect L2 activity response or not, I artificially activated R8 via TRPA1 (Transient receptor potential cation channel A1). TRPA1 is a thermosensitive cation channel that can be activated by warming (Luo et al., 2017). I found that the histamine receptor level in the medullar part of L2 was decreased upon R8 activation (Fig. 17). This result indicates that activating R8 is sufficient to induce histamine receptor level downregulation in the medullar part of L2 under continuous light conditions.

7. L2 histamine receptor level is regulated by L2 activity through CaMKII-related pathway

To confirm that HR downregulation in L2 is activity-dependent, I artificially activated L2 using TRPA1 under 29 °C and found that HR loss was enhanced (Fig. 18). According to previous studies, *Drosophila* neuromuscular junction and *Drosophila* olfactory system all require CaMKII in activity-dependent synaptic plasticity. Moreover, studies in the rodent hippocampus demonstrate the involvement of CaMKII-related pathway and CREB-B in activity-dependent postsynaptic modification. Knockdown of CaMKII in L2 with CaMKII-RNAi highly suppressed the decrease of HRs after 6-day LL. Overexpression of CREB-B with UAS-CrebB in L2 also highly suppressed the decrease of HRs in L2 under both continuous LL and DD conditions, and the phenotype just resembled expressing CaMKII-RNAi in L2 (Fig. 19A). Knockdown of CREB-B using CrebB-RNAi enhanced the decrease of HRs in L2 after both 6-day LL and DD conditions (Fig. 19B). Since CaMKII is confirmed to be a negative CREB-B regulator (Matthews et al., 1994; Wu and McMurray, 2001; Carlezon et al., 2005), calcium influx in L2 stimulates CaMKII, and then CaMKII suppresses CREB-B, in result downregulating the expression of HRs in L2. These findings indicate that the CaMKII-related pathway is involved in the activity-dependent transcriptional HR downregulation in L2.

8. Exogenous expression of Ort in L2 neurons attenuates the enhanced activity response caused by constant light exposure

Next, using UAS-ort, GAL80ts, and the TRIC system, we expressed Ort proteins in L2 neurons on day 7 after 6-day LL treatment and monitored the activity responses of L2 toward 1-day photoreceptor activation simultaneously. The results showed significantly fewer L2 neurons in flies with exogenous expression of Ort in L2 on day 7 after 6-day LL treatment (an average of 2.57 neurons within a 15-μm range of depth) exhibiting TRIC GFP signals after 1 day of photoreceptor activation compared to flies in the control groups treated with 6-day LL and 1-day photoreceptor activation (an average of 7.73 neurons within a 15-μm range of depth) (Fig. 20). This result demonstrates that exogenous expression of Ort in L2 on day 7 reverted the activity response change caused by

prolonged light exposure. Along with the finding that there was transcriptional downregulation of HRs in L2 after 6-day LL treatment, this result supports our hypothesis that the change in L2 activity response to 1-day photoreceptor activation after prolonged light exposure is derived from HR reduction.

9. Histamine receptor downregulation does not occur in L1 upon prolonged light exposure

According to the TRIC experiment, it shows that after prolonged light exposure, only some specific subsets of postsynaptic neurons such as L2 neurons increase their activity responses upon photoreceptor activation. Even though lamina monopolar neuron L1 also has connections with photoreceptors, L1 neurons do not show enhanced activity responses. To assess whether the HR downregulation occurring in L2 after 6-day LL also happens in L1 or not, I checked the HR expression level specifically in L1 and found that there was no significant difference in OLLAS staining intensity in L1 between 6-day LL and 6-day DD (Fig. 21A). Reverse transcriptional PCR and Real-time PCR were also applied to examine the mRNA level of OLLAS-tagged HR specifically in L1. Results showed that the transcriptional level of OLLAS-tagged HR was not changed after 6-day LL treatment (Fig. 21B). It reveals that there is no downregulation of HR in L1 upon prolonged light exposure and this may be the reason why activity response changes happen only in L2, not in L1, in the TRIC assay.

10. Activation of L2 stabilizes the synapse number in R8

In a previous study, double mutants in histamine with ort^{1} $hiscl^{1}$ suppressed delocalization of presynaptic components (Sugie et al., 2015). There is a possibility that activation of L2 through HR downregulation participates in the retrograde signaling process to stabilize the presynaptic structure in R8 photoreceptors. I artificially activated L2 via TRPA1 and counted the synapse numbers. In the control group without L2 activation, synapse number in R8 photoreceptor decreased significantly on average from 33.42 to 29.28 in one axon. In the experiment group with L2 activation, results showed that R8 photoreceptors lost fewer synapses compared to the control group (Fig. 22). It indicated that activation of L2 suppressed the activity-dependent presynaptic modification in R8, which implies the biological significance of postsynaptic receptor downregulation and activity change.

4. Discussion

1. Circuit responses upon photoreceptor activation change after various light conditions in specific subsets of postsynaptic neurons

In this study, I applied the TRIC method, expressing GFP in neurons postsynaptic to photoreceptors when there is calcium influx, to achieve a better understanding of the subsequent influence of activity-dependent, environmental stimulation-induced synaptic plasticity in the *Drosophila* visual system. The TRIC validation of the physiological response showed that only some subsets of postsynaptic neurons, such as L2 neurons, exhibit a more robust activity response to environmental stimulation after chronic light treatment-induced activity-dependent synaptic plasticity in the *Drosophila* visual system. L1 neurons also have connections with photoreceptors as L2, but their activity responses have not changed. This research succeeds to visualize the circuit change in a selective manner after activity-dependent synaptic plasticity evoked by long-term activation.

2. Enhanced L2 neuronal activity response is a part of the adaption to prolonged light exposure

In photoreceptors, ambient light exposure reportedly induces the dominant arrestin isoform Arr2, which is translocated to the rhabdomere loaded with R1–6 and inactivated photoreceptor response (Satoh et al., 2010). Moreover, recent studies indicated that continuous light conditions trigger photoreceptor neuronal component reorganization in AZ through microtubule destabilization and reduction of the number of synapses (Sugie et al., 2015). Such processes allow *Drosophila* visual system to adapt to prolonged light exposure. In this research, I propose that the enhanced L2 neuronal activity response is also a part of the adaption to prolonged light exposure. Since L2 neurons reveal significant selective calcium signals in response to light OFF (decrement) under physiological situations (Joesch et al., 2010), enhanced L2 neuronal activity responses may allow flies to respond to subsequent light changes more efficiently and faster.

3. Postsynaptic modification involving transcriptional regulation of neurotransmitter receptors is responsible for circuit changes

Since the presynaptic remodeling with a slight synapse number reduction is insufficient to account for the activity-dependent, synaptic plasticity-induced robust circuitry response sensitivity change in the postsynaptic neurons, I aimed at searching for further molecular mechanisms underlying this phenomenon in the postsynaptic terminal. I successfully observed a reduced histamine receptor protein expression and mRNA level in the medullar part of the L2 neurons under LL conditions. Previous studies showed that the postsynaptic modification during activity-dependent synaptic plasticity could occur mainly through regulation of postsynaptic receptor localization and recycling. A recent study demonstrated the transcriptional regulation of receptors during the developmental stage in the visual circuit of *Drosophila* (Yin et al., 2018). Another recent study in the glial cells of adult *Drosophila* demonstrated that transcriptional regulation of the glutamate/GABA/glutamate cycle could control the neuronal neurotransmitter levels and behavior output (Mazaud et al., 2019). In this study, I propose that the histamine receptor downregulation in adult *Drosophila* postsynaptic L2 neurons is activity-dependent and occurs on the transcriptional level. Like postsynaptic modifications in the rodent hippocampus, the histamine receptor transcriptional downregulation is also regulated by CaMKII and CREB-B.

HR downregulation occurs only in L2 after 6-day LL, not but in L1, which is consistent with the observation that only L2-like neurons showed increased activity response to photoreceptor activation after prolonged light exposure. These findings together with the fact that histamine is the main and inhibitory neurotransmitter released by photoreceptors in the *Drosophila* visual system strengthen the hypothesis that the activity-dependent transcriptional downregulation of the L2 HRs is partially responsible for the activity-dependent synaptic plasticity-induced circuitry response change. After the visual circuit of *Drosophila* receives long-term activation, synaptic plasticity involving activity-dependent transcriptional downregulation of HRs occurs specifically in L2 neurons and then changes the activity responses of L2 upon subsequent photoreceptor activation. (Fig. 23)

4. Transcriptional regulation of histamine receptors in postsynaptic neurons may also participate in the feedback to regulate photoreceptor function

In a previous report, histamine-deficient *hdc*^{JK910} mutant photoreceptors showed more moderate responses to prolonged light stimulation than the wild-type photoreceptors, but their information transfer and adaptation were close to the physiological level due to the excitatory feedback from depolarized (in the absence of inhibitory histaminergic input) postsynaptic interneurons (Dau et al., 2016). Interestingly, at the *Drosophila* neuromuscular junction, postsynaptic (glutamate) receptor reduction leads to presynaptic transmitter release changes, indicating a postsynaptic activity-induced retrograde signal regulating presynaptic function, also involving calcium influx in the postsynaptic terminal and CaMKII (Petersen et al., 1997; Haghighi et al., 2003; Kazama et al., 2003). As revealed in the L2 artificial activation experiment, increased L2 activity can stabilize the location of presynaptic components in R8 photoreceptors. The HR downregulation caused by L2 activity further amplifies the calcium influx in L2, in return suppressing the synapse loss in presynaptic photoreceptor, which is consistent with the finding in a previous study that HR double mutants can elicit delocalization of presynaptic components (Sugie et al., 2015). Depolarization in L2 is confirmed to have effects on the photoreceptor.

This study revealed the possibility that the transcriptional regulation of histamine receptors in neurons postsynaptic to photoreceptors might also participate in the feedback to regulate photoreceptor function and vision.

5. Sparse connections exist between photoreceptor R8 and L2 in medulla

In a previous study, the electron microscopy-assisted connectome reconstruction showed that R8 photoreceptors turned out to exhibit no synaptic connection with L2 in the medulla (Takemura et al., 2013), which was in contrast with my observations. I found synaptic connections between R8 and the L2 neurons in medulla using activity-dependent GRASP and trans-Tango. The differences in our findings could be due to the sparse number of synapses between R8 and L2 since not all R8 photoreceptors seemed to maintain connections with the L2 neurons in my results. The reference column and adjacent medullar columns chosen for electron microscopic reconstruction in the previous

study might not include those sparse synaptic connections between R8 and L2. (Takemura et al., 2011; Takemura et al., 2013)

6. R1–6 and R8 activities account for the L2 histamine receptor level regulation

The results of *ninaE* mutation experiment showed that R1–6 activities are necessary for the histamine receptor level regulation in the L2 neurons. R1–6 photoreceptors maintain synaptic connections with L2 neurons in the lamina and L2 neurons mainly receive light information input from R1–6. Therefore, R1–6 photoreceptor activity appears to account for significant histamine receptor downregulation in the L2 neurons. Meanwhile, I found synaptic connections between R8 and L2 in the medullar part, and activating R8 was sufficient to downregulate L2 histamine receptors, suggesting the possibility that the L2 histamine receptor level is regulated by multiple factors. R1–6- and R8-related regulatory mechanisms are activity-dependent in both cases. In this study, it was also interesting that among all the neurons postsynaptic to photoreceptors, only a small portion of certain neuron types, such as L2 neurons, showed robust response change to activity-dependent synaptic plasticity in the photoreceptors. This particularity might imply the special role of the L2 neurons in integrating light-sensing, motion detection, and color vision, consistently with the central place of L2 as secondary neurons in the medullar visual circuit.

7. Time course of activity and histamine receptor regulation in L2

Results showed that histamine receptor downregulation in the postsynaptic L2 neurons started not earlier than 3 days after eclosion, which was consistent with the fact that the 3-day LL condition was not enough to cause increased L2 activity response to photoreceptor activation. The reason why HR downregulation and postsynaptic circuitry response change started after 3-day remains to be explained. The mechanism underlying the baseline activity of L2 is also unclear. It is broadly believed that the main input that L2 neurons directly get from photoreceptors is inhibitory since photoreceptors mainly release histamine. However, there is a baseline calcium influx in L2, which seems to be contradictive. In a recently published study, R8 photoreceptors reportedly release not only inhibitory histamine but also excitatory acetylcholine to the postsynaptic neurons and

indirectly activate the L2 neurons (Davis et al., 2020). It implies the possibility that upon light stimulation L2 is hyperpolarized at first but goes up back to a slight depolarized state as the baseline activity after a certain period. This might partially explain why there is a slight calcium influx in L2 under light conditions even if the main input that L2 neurons directly get from photoreceptors is inhibitory. Therefore, there may be a threshold of activity for L2, and only after 3-day LL can calcium influx surpass the threshold and trigger any further changes in the circuit. A previous report also demonstrated that the synapse number in photoreceptors was significantly reduced after a constant 3-day light exposure after eclosion in an activity-dependent manner (Sugie et al., 2015). This finding strongly supports the requirement of a 3-day LL to pass the activity threshold. It also means that a synapse number decrease in the photoreceptors takes place prior to HR downregulation in the postsynaptic neurons. Upon long-term light exposure, from day 3, the increased calcium influx in L2 triggers HR downregulation. The reduced HR level in the constitutively active L2 neurons attenuates the inhibitory input directly from the photoreceptors and further enhances calcium influx in L2. Therefore, L2 neurons with less HR can respond more drastically to the photoreceptor stimulation after the LL condition (Fig. 24). Hereby, I propose the model that upon chronic light treatment-induced synaptic plasticity in *Drosophila*, due to the postsynaptic inhibitory histamine receptor transcriptional downregulation, depended on the activity of photoreceptors and postsynaptic neurons, the postsynaptic neurons in the medulla showed more robust activity responses to subsequent environmental stimulation (Fig. 25).

8. L1 neurons act differently from L2 in the first 3 days

It is interesting that HR downregulation and activity response change happen in L2 neurons, but not L1. There should be some differences between L1 and L2 neurons. According to the time course I have illustrated in the last paragraph, since L1 did not show HR downregulation which happened between day 3 to day 6 in L2, and no increased activity response on day 7, the branching point of L1 and L2 performances should occur before day 3. I supposed that the response of L1 to light stimulation at the very beginning may account for the phenomenon. Photoreceptors can release not only inhibitory neurotransmitter histamine but also excitatory neurotransmitter acetylcholine to the postsynaptic neurons. L2 neurons express a high level of excitatory acetylcholine receptors while L1 neurons express much less, and L2 neurons also express a relatively higher level of excitatory GABA-A receptors than L1 (Davis et al., 2020). It implies that

when there is light stimulation at the very beginning, L1 neurons get inhibitory input, less excitatory input, and as a result more intense hyperpolarization than L2 neurons and it may take a much longer time for L1 neurons to go back to baseline activity. Thus, for the first 3 days, L1 neurons may experience long hyperpolarization status and fail to trigger further presynaptic modification, HR regulation or activity response change like L2 (Fig. 26).

9. Regulation relationship among R1–6, R8, and L2

The R1–6-mediated regulation of L2 in the lamina during LL induced histamine receptor downregulation in all L2 neurons, thereby further affecting the connections between R8 and L2 in the medulla, changing the information flow of the circuits in an activity-dependent manner, which hinted the biological significance of whole regulation. It implied the existence of a complicated type of regulatory relationship among R1–6, postsynaptic L2, and R8, and the role of L2 in this "crosstalk" of light-sensing, motion detection, and color vision under prolonged light exposure remains to be unraveled. In a brighter place, human eyes are most sensitive to the yellow-green wavelengths (555 nm), while in a darker place, most sensitive to blue-green wavelengths (505 nm). Moreover, if one has sunbathing without sunglasses for only 4.5 hours, color vision of blue and green will be disturbed, which can persist for 7 months (Spalding, 1999). The functions of light-sensing and color-sensing seem to correlate to each other tightly. The findings of this research suggest the possibility that there may be simultaneous regulation of the light- and color-sensing circuits in the *Drosophila* visual system. (Fig. 25)

Hereby, I report that in the adult *Drosophila* visual system, after constant light exposure-induced synaptic plasticity, inhibitory neurotransmitter histamine receptor transcriptional downregulation occurs in the postsynaptic lamina neuron L2, but not L1, depending on the photoreceptor and postsynaptic neuronal activity and involving CaMKII and CrebB. The histamine receptor transcriptional downregulation results in a more intense postsynaptic L2 neuronal response to subsequent environmental stimulations, especially in the medulla. This research succeeded to visualize the circuit change process after synaptic plasticity according to long-term activation and find in vivo evidence of the circuitry plasticity upon long-term environment stimulation. Findings in this research deepen the understanding of the consequence of activity-dependent synaptic plasticity to the circuit and provide new insights into the activity-dependent synaptic plasticity

mechanism in the neural circuit.

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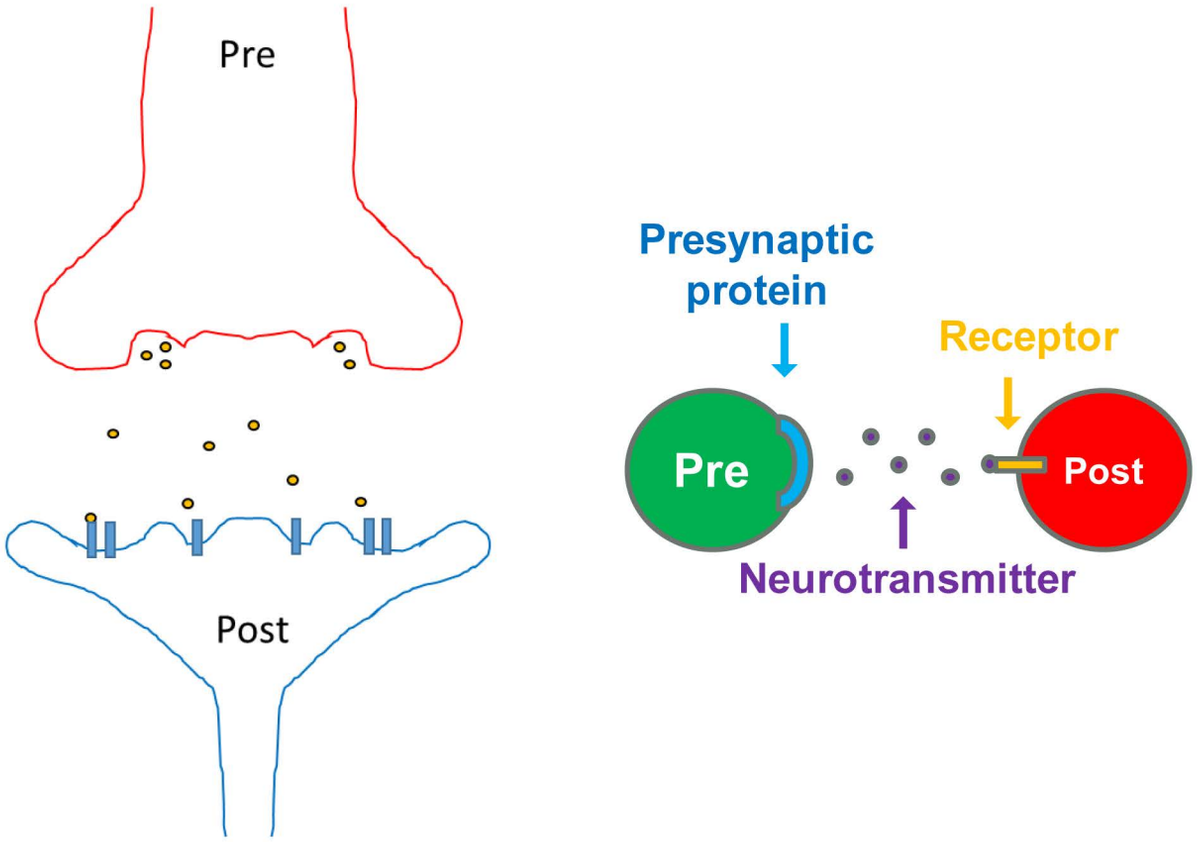
Figure 1

A



B

Cellular/Morphological scale	Subcellular/Molecular scale
Neurogenesis	Synaptic component expression
Wiring pattern	Neurotransmitter level
Dendritic branching	Receptor expression

Fig.1 Synapse and plasticity

A. Illustration for synapse.Synapses allow presynaptic neuron to pass a chemical or electrical signal to postsynaptic neuron. For chemical synapses, presynaptic neurons will release chemical substances called neurotransmitters which are contained in synaptic vesicles. Postsynaptic neurons can receive neurotransmitters via corresponding neurotransmitter receptors and trigger responses.

B. The plasticity occurring during adaption at different scales. In cellular, or morphological scale, neurogenesis, dendritic branching and wiring pattern can be modified. In subcellular, or molecular scale, neurotransmitter level, receptor expression, synaptic component expression and various factors can be adjusted upon different environmental experiences.

Figure 2

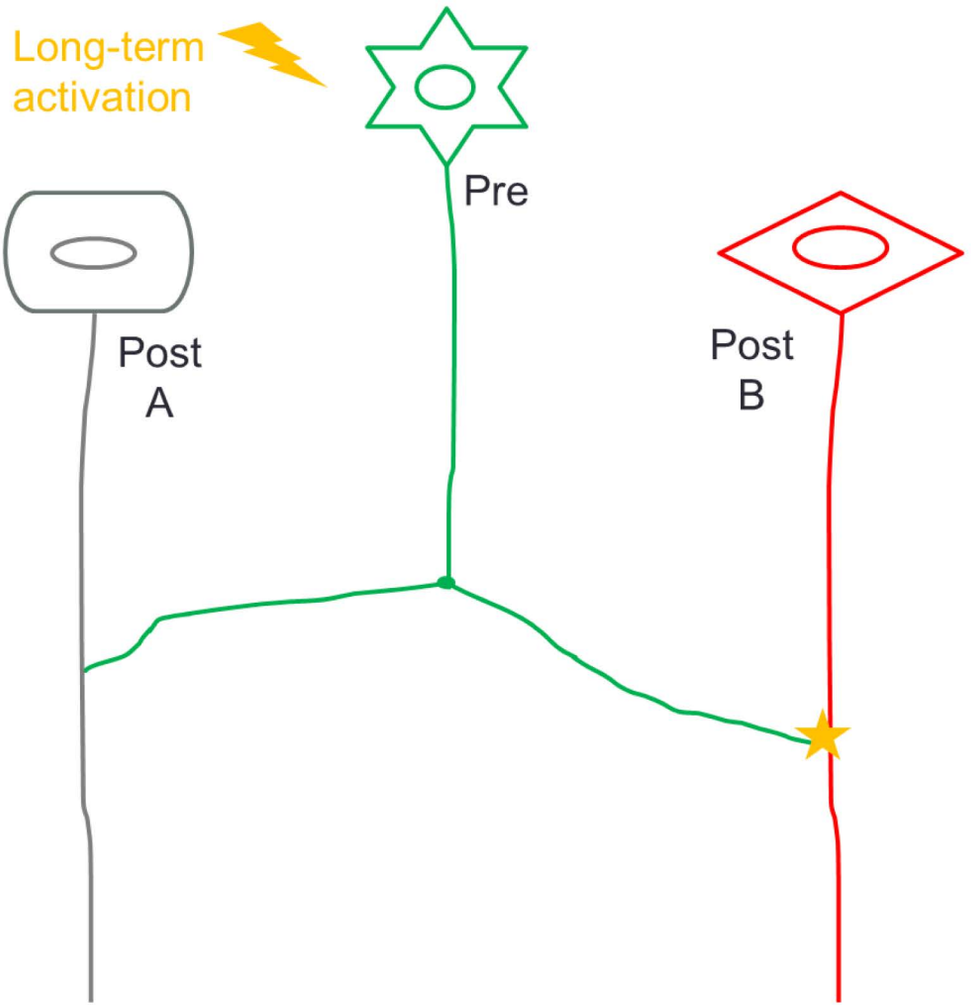


Fig.2 Circuit change upon long-term activation

When a circuit receives input activity, direct synaptic changes occur and have subsequent consequences in the circuit. One type of presynaptic neuron usually has connections with various types of postsynaptic neurons.
It remains a big question whether synaptic plasticity happens among all the connections, or only some specific subsets of postsynaptic neurons change the connections with the presynaptic neuron and modify their activity responses according to input activity.
For example, postsynaptic neuron A and postsynaptic neuron B both are postsynaptic to one presynaptic neuron. Under long-term environment stimulation, only some subsets of postsynaptic neurons, for example, postsynaptic neuron B will occur plasticity change according to the activation. This research is meant to visualize this circuitry change process and find in vivo evidence of the circuitry plasticity upon long-term environment stimulation.

Figure 3

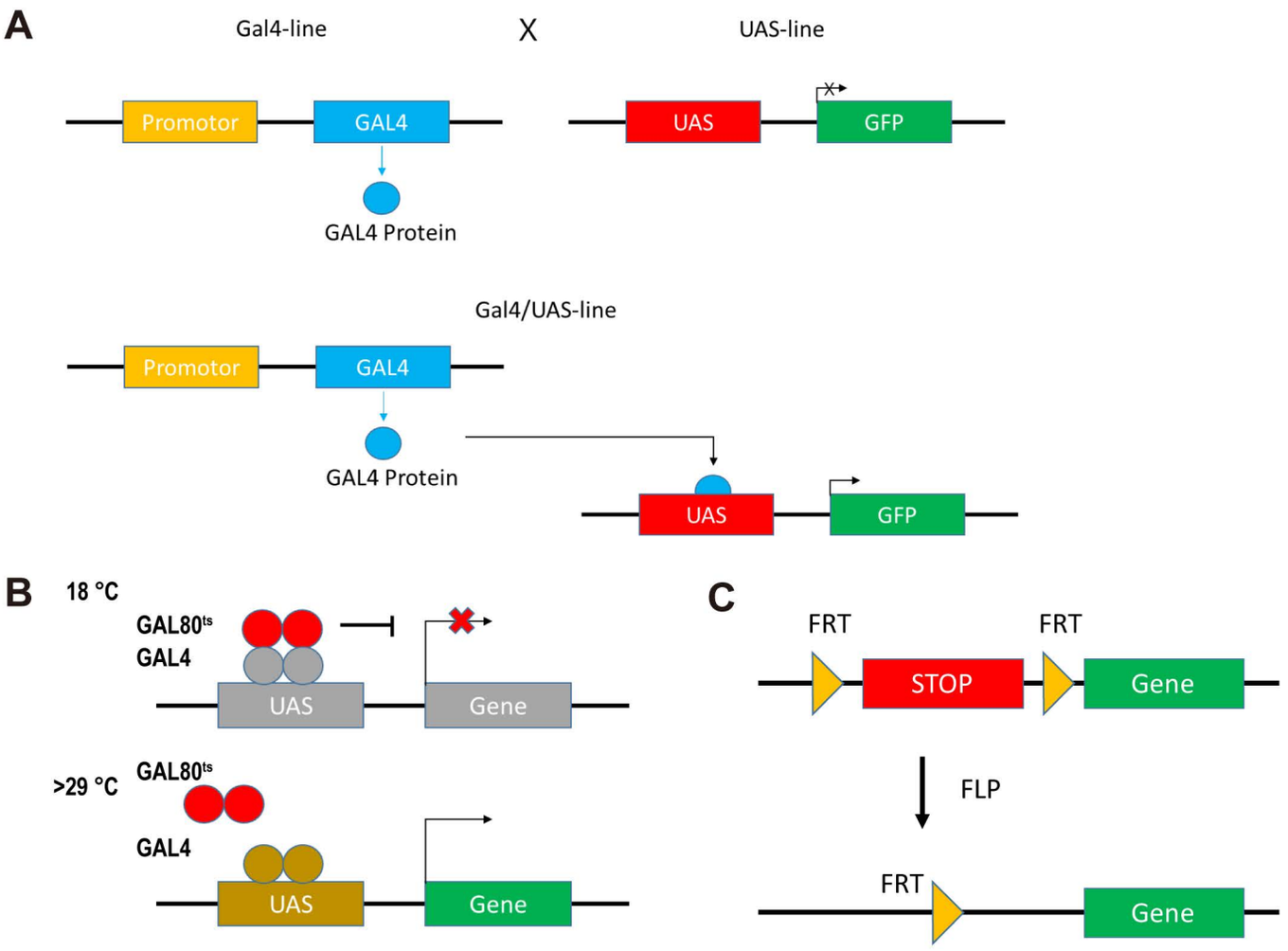


Fig.3 Commonly-used genetic tools in *Drosophila*

A. Gal4-UAS system. Gal4 protein can specifically bind to UAS to trigger the gene transcription in the downstream.

B. Gal80^{ts} (temperature-sensitive version of the GAL80 protein). Gal80^{ts} allows Gal4-UAS functioning at the temperature over 29 °C, and represses Gal4 at 18 °C.

C. FRT-STOP-FRT (FRT-flanked transcriptional stop cassette). FRT-STOP-FRT cassette contains transcriptional stop sequence flanked by two FRT sites. With cell-specific FLP construct, or neuron-specific Gal4 lines and UAS-FLP, the FRT-STOP-FRT cassette can be removed in specific subsets of neurons, which in result allows expression of target genes in the downstream.

Figure 4

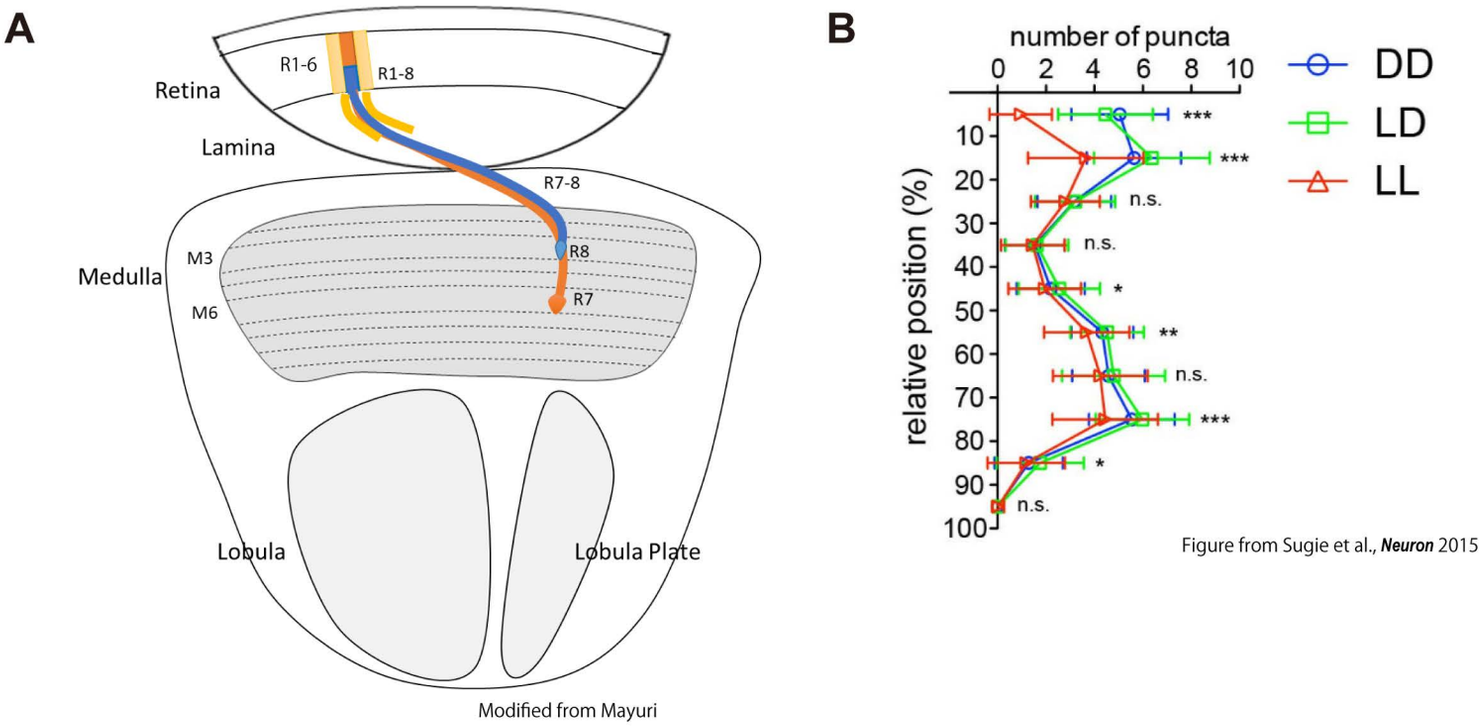


Fig.4 *Drosophila* visual system and activity-dependent synaptic plasticity

A. Illustration for *Drosophila* visual system. The adult *Drosophila* visual system comprises retina and optic lobe. The optic lobe consists of lamina, medulla, lobula and lobula plate. There are estimate 750 small eyes called ommatidia in retina and each ommatidium has eight types of photoreceptor neurons (R1 to R8).
R1–6 photoreceptors project to lamina, while R7 and R8 photoreceptors extend to M6 and M3 layer of medulla respectively.
B. Activity-dependent synaptic plasticity in *Drosophila* visual system. Photoreceptor R8 lost synaptic connections with its postsynaptic neurons after continous light condition (LL), especially in M2 layer of medulla.
(Sugie et al., *Neuron* 2015)

Figure 5

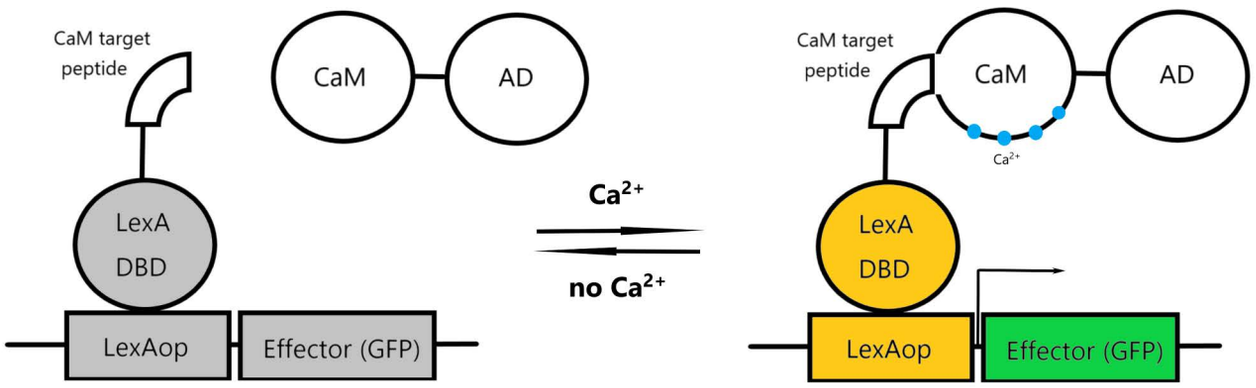


Fig.5 Transcriptional reporter of intracellular calcium (TRIC)

Scheme of TRIC. TRIC (Transcriptional Reporter of Intracellular Ca^{2+}), is a new tool to monitor changes in calcium levels over long periods of time with a split binary expression system. When calcium influx occurs, calmodulin interacts with its target peptides, and allows subsequent expression of reporter genes through the LexA/LexAop system.

Figure 6

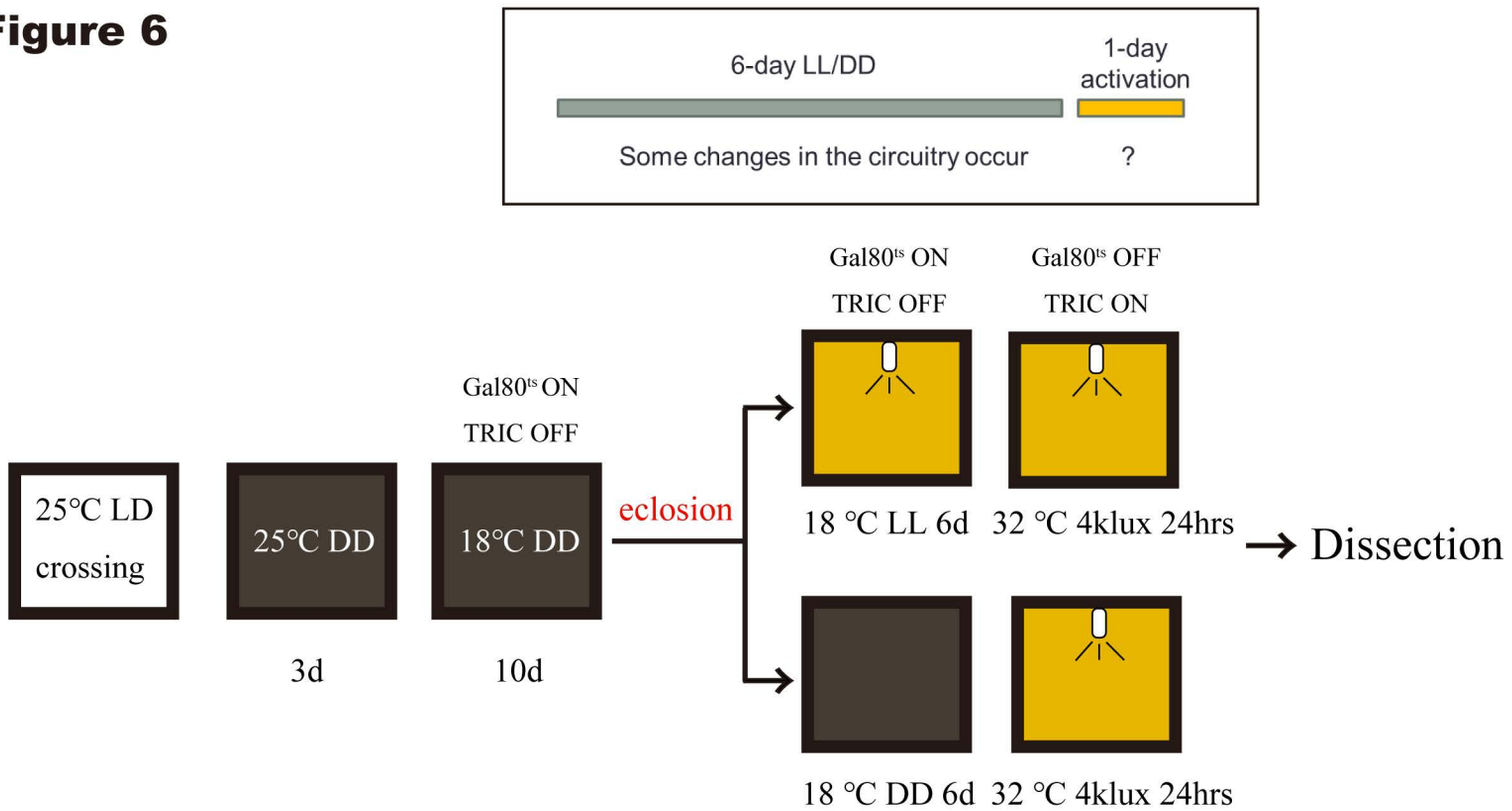


Fig.6 Experiment design for TRIC assay

After 2-day crossing in 25 °C, on day 3, adult flies were taken away, and vials were covered by foil paper, placed in 25 °C for 2 days.

On day 5, the vials were moved to a 18 °C incubator.

On day 15, flies were collected and divided into two groups to be treated by LL or DD for 6 days under 18 °C after eclosion.

One day continuous light condition under 32 °C was applied on the 7th day, and fly brains were dissected to check the circuitry response towards this 1-day photoreceptor activation.

Figure 7

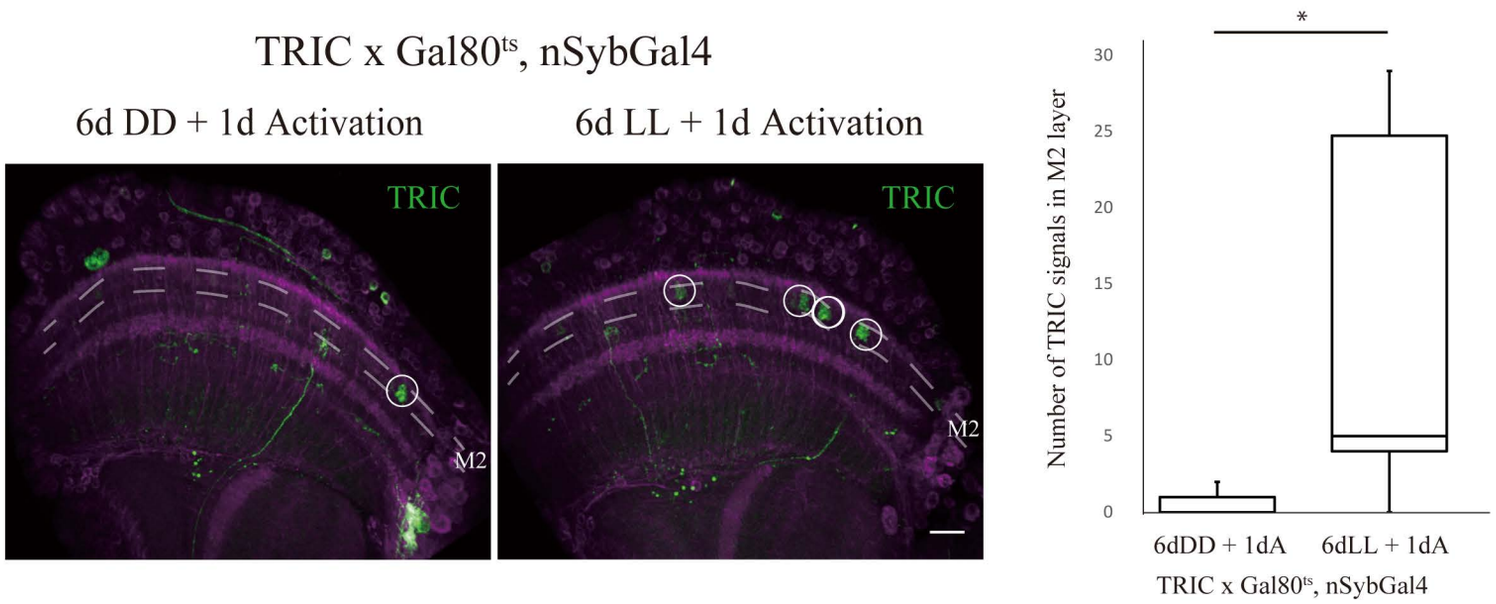


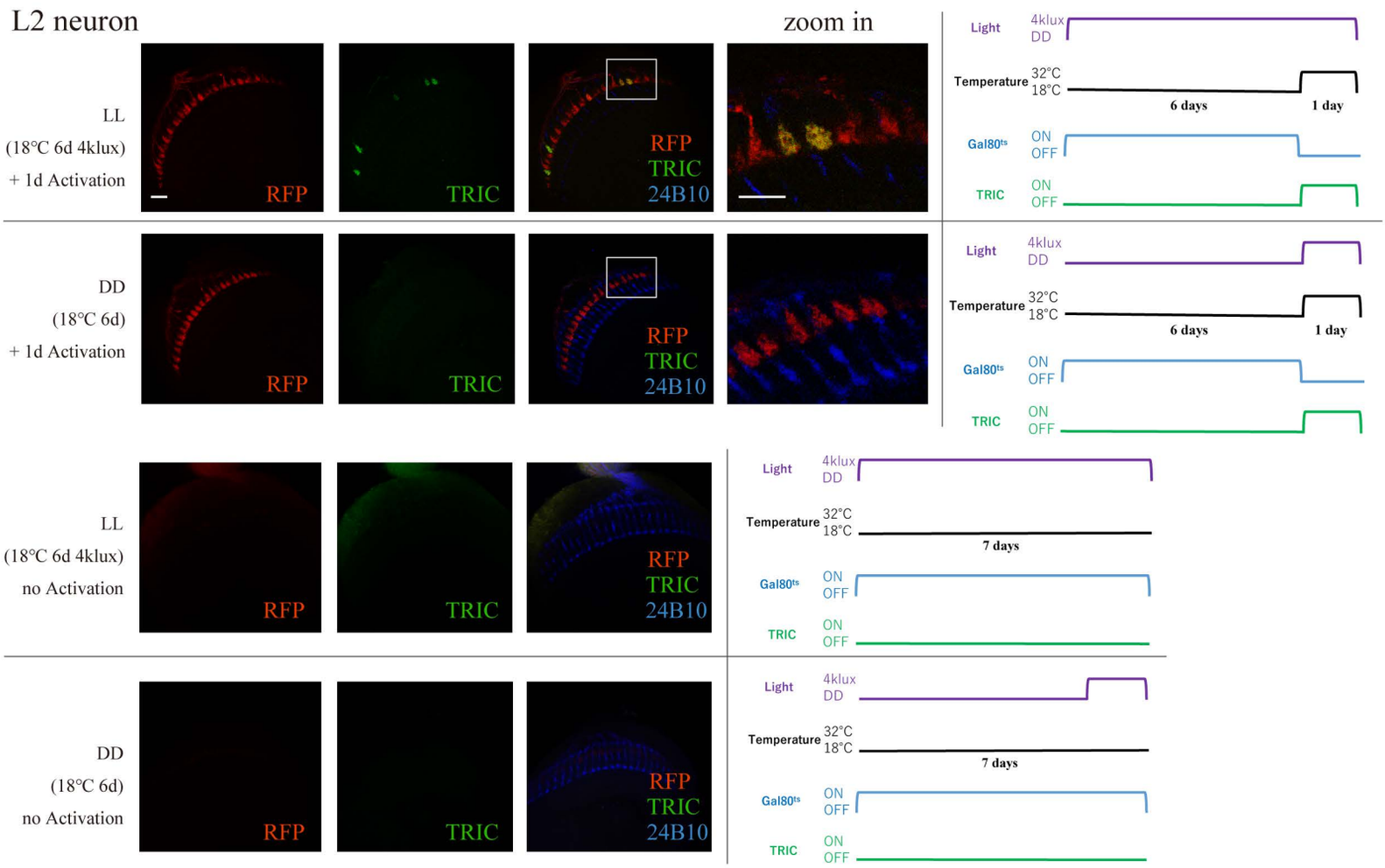
Fig.7 Pan-neuronal TRIC assay

Pan-neuronal TRIC signals in visual circuit for 1-day activation after 6-day LL or DD.

Some neurons with oval structure in medulla (white circle), mostly like lamina monopolar neuron L2, showed significantly more TRIC GFP signals with step size of 0.5 μm in a sample cross section size of 15μm after 1-day activation subsequent to 6-day LL treatment (12.5 neurons with oval structure in medulla in average) than the ones treated with 6-day DD (0.44 neurons with oval structure in medulla in average). Scale bar 10 μm.

Student’ s t-test, LL: n=10; DD: n=9. p=0.0102.

Figure 8



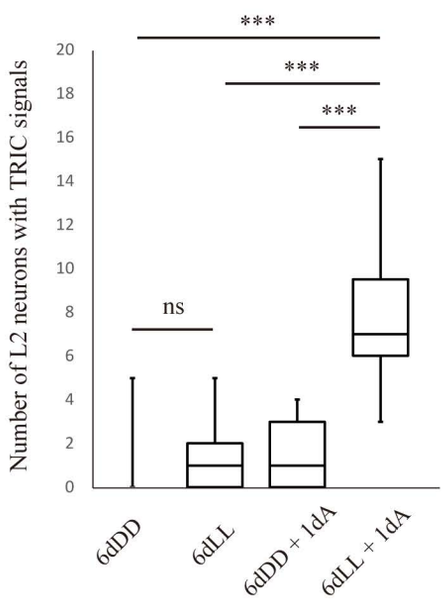


Fig.8 TRIC assay in lamina monopolar neuron L2

TRIC assay specifically performed in L2.

TRIC GFP signals in L2 after 1-day activation subsequent to 6-day LL or DD. Images were taken using NIS-Element AR (Nikon). The status of light temperature, Gal80ts and TRIC expression under different conditions are shown beside the confocal images.

There were significantly more L2 neurons in flies treated with 6-day LL condition with step size of 0.5 μm in a sample cross section size of 15 μm showing bright TRIC signals after activation than the ones treated with 6-day DD condition. Scale bar 10 μm.

Statistical tests between groups: $p<0.00001$, Kruskal-Wallis test; and ***$p<0.001$, post hoc Mann-Whitney U test.

Figure 9

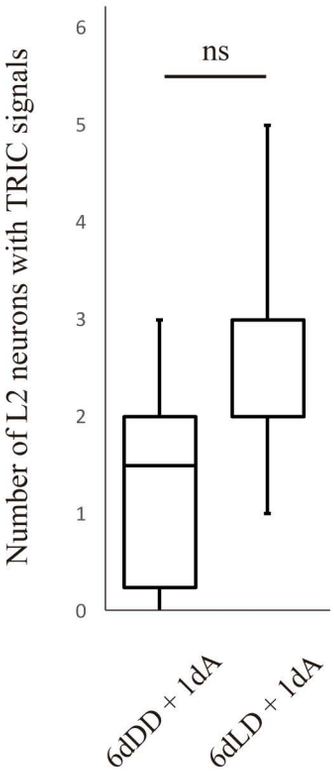


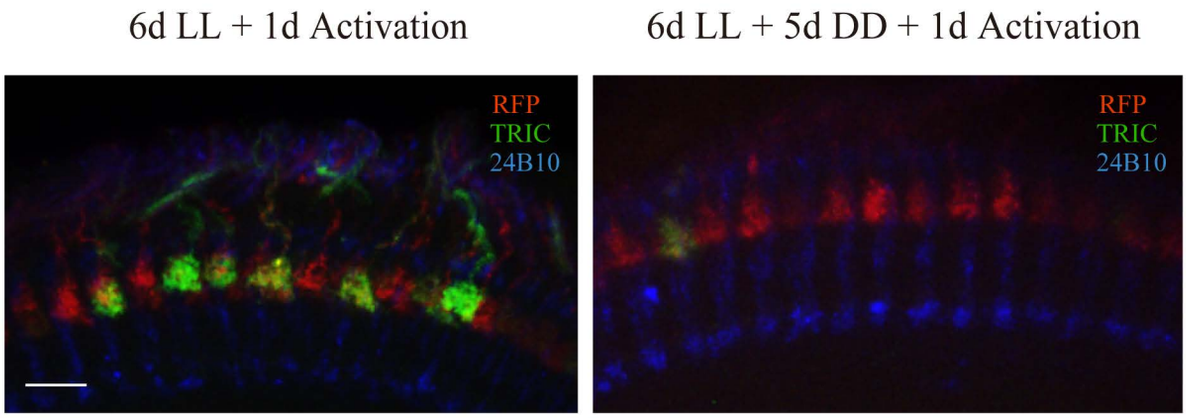
Fig.9 DD condition had no significant effect on the activity response

Flies treated with 6-day LD condition showed no significant difference in the number of TRIC-positive L2 neurons after 1-day activation compared with those treated with 6-day DD.

Student’ s t-test, 6-day DD + 1-day activation: n=6, avg=1.33;

6-day LD + 1-day activation: n=5, avg=2.8. *p*=0.115.

Figure 10



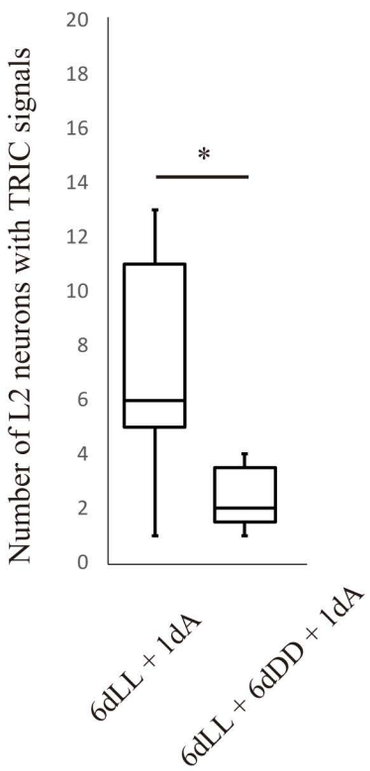


Fig.10 Reversibility of circuit response change

Response difference caused by continuous light treatment in L2 is reversible.

Scale bar 10 μm.

Student' s t-test, 6-day LL + 1-day activation: n=9, avg=7;

6-day LL + 6-day DD + 1-day activation: n=7, avg=2.43. *p*=0.0119.

Figure 11

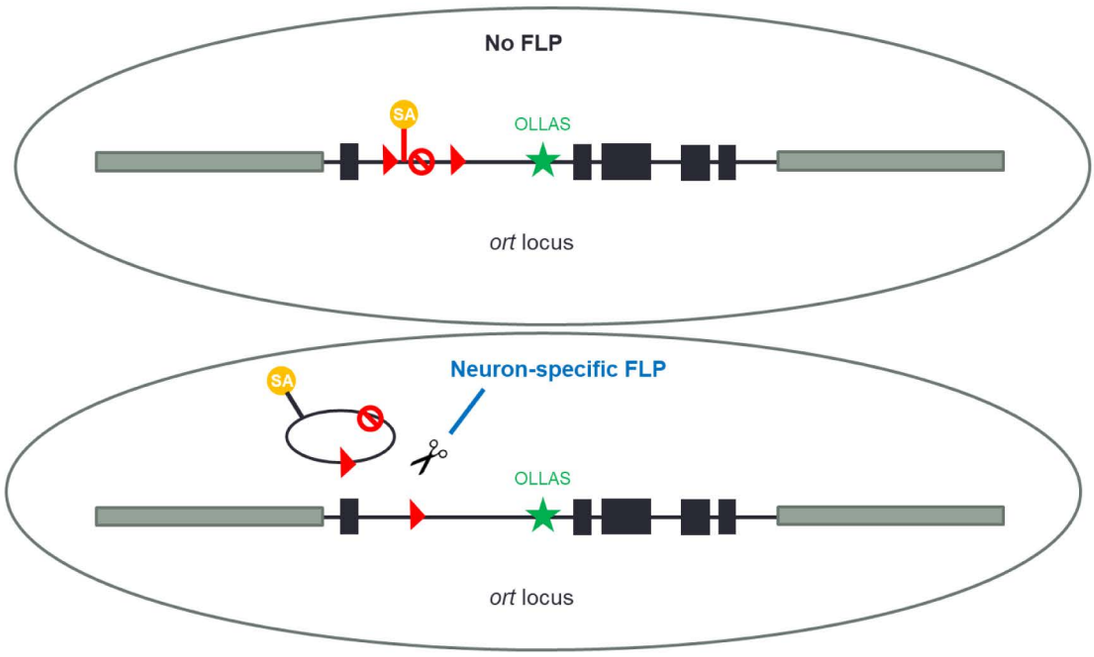


Fig.11 Neuron-type-specific expression of OLLAS-tagged ORT proteins

OLLAS (OmpF Linker and mouse Langerin fusion Sequence) is used as a tag linked with ORT protein (HR protein in postsynaptic neurons of photoreceptors) to label the HR. To achieve neuron-type-specific expression of tagged ORT proteins, FRT-flanked transcriptional and translational stop cassette with a splice acceptor (SA) was inserted in the upstream of OLLAS-tagged Ort gene. Only when combined with a neuron-type-specific FLP recombinase can the stop cassette be removed, allowing the expression of OLLAS-tagged ORT protein. Modified from Chen et al., 2014.

Figure 12

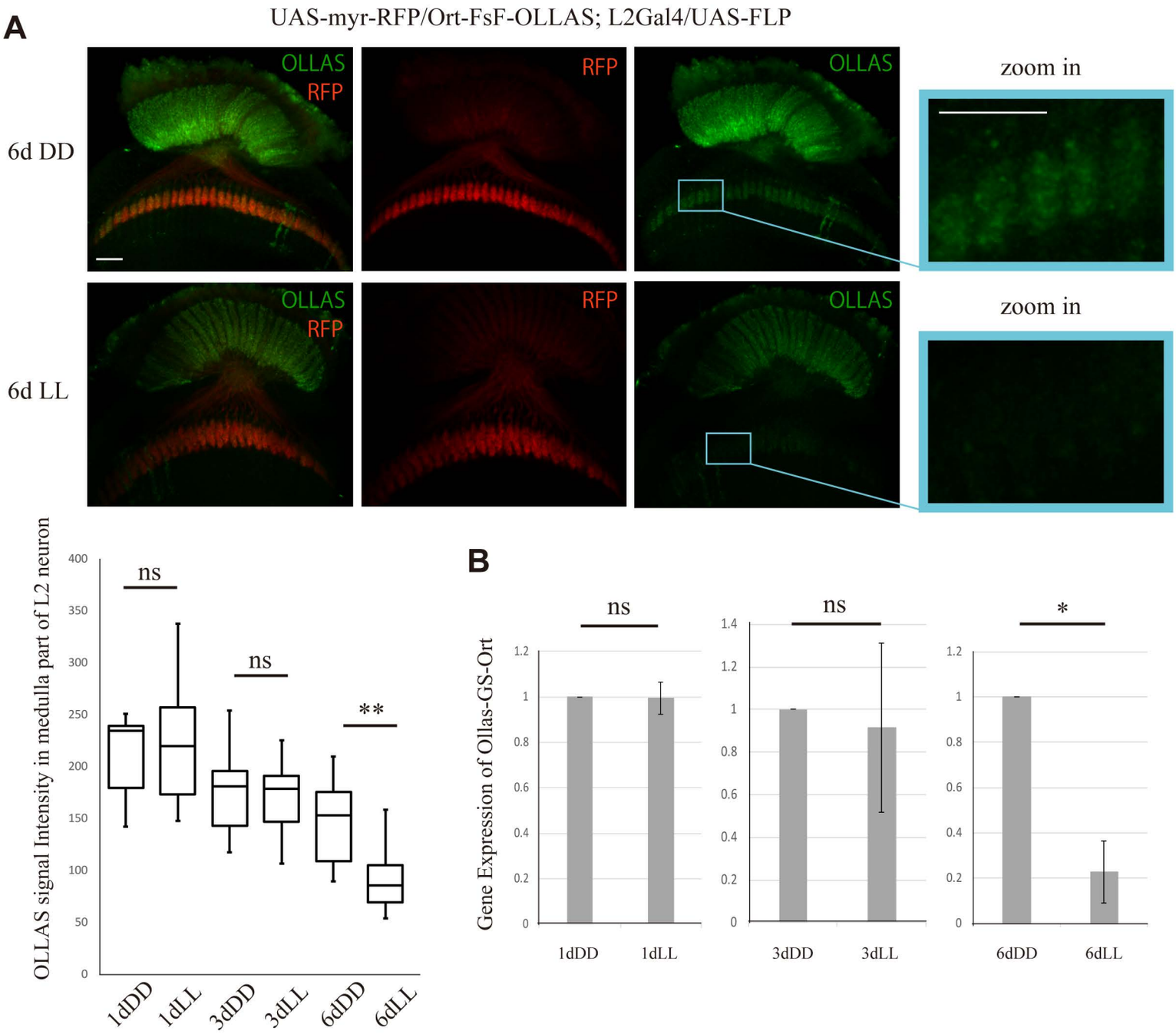


Fig.12 Transcriptional downregulation of histamine receptor in L2

Transcriptional downregulation of histamine receptor in L2 neurons after prolonged light exposure.

A. Immunostaining using an anti-OLLAS antibody (green) of Drosophila brains after 6-day LL or DD. Scanning was done with step size of 0.5 μm in a sample cross section size of 15 μm. The intensity of OLLAS signal of L2 in medulla was significantly decreased under 6-day LL condition compared to 6-day DD, while there was no significant decrease after 1-day or 3-day LL. Scale bar 10 μm.

Statistical tests between groups: p=0.00004313, Kruskal-Wallis test; and **p<0.01, post hoc Mann-Whitney U test.

B.Three independent biological replicates and several technical replicates for each biological replicates were measured for LL or DD condition. Housekeeping gene Rpl32 was used as reference.The calculation of threshold cycle (Ct) of qPCR followed 2nd Derivative Maximum method. Relative quality of LL is calculated compared to DD (defined as 1). Student’ s t-test, 1-day LL: 0.995; 3-day LL: 0.914; 6-day LL: 0.228; p=0.954(1dDD–1dLL), p=0.848(3dDD–3dLL), p =0.0298(6dDD–6dLL).

Figure 13

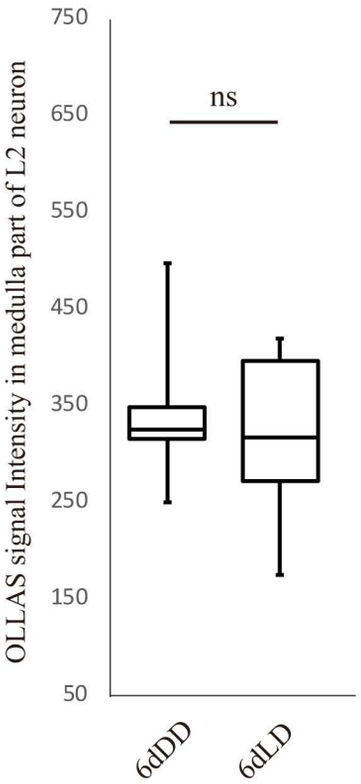


Fig.13 DD condition had no significant effect on HR decrease in L2

Flies treated with 6-day LD condition showed no significant difference in the HR level in L2 neurons compared with those treated with 6-day DD.

Student' s t-test, 6-day DD: n=6, avg=342.08;

6-day LD: n=5, avg=313.8. p=0.625.

6dDD 6dLL

Figure 14

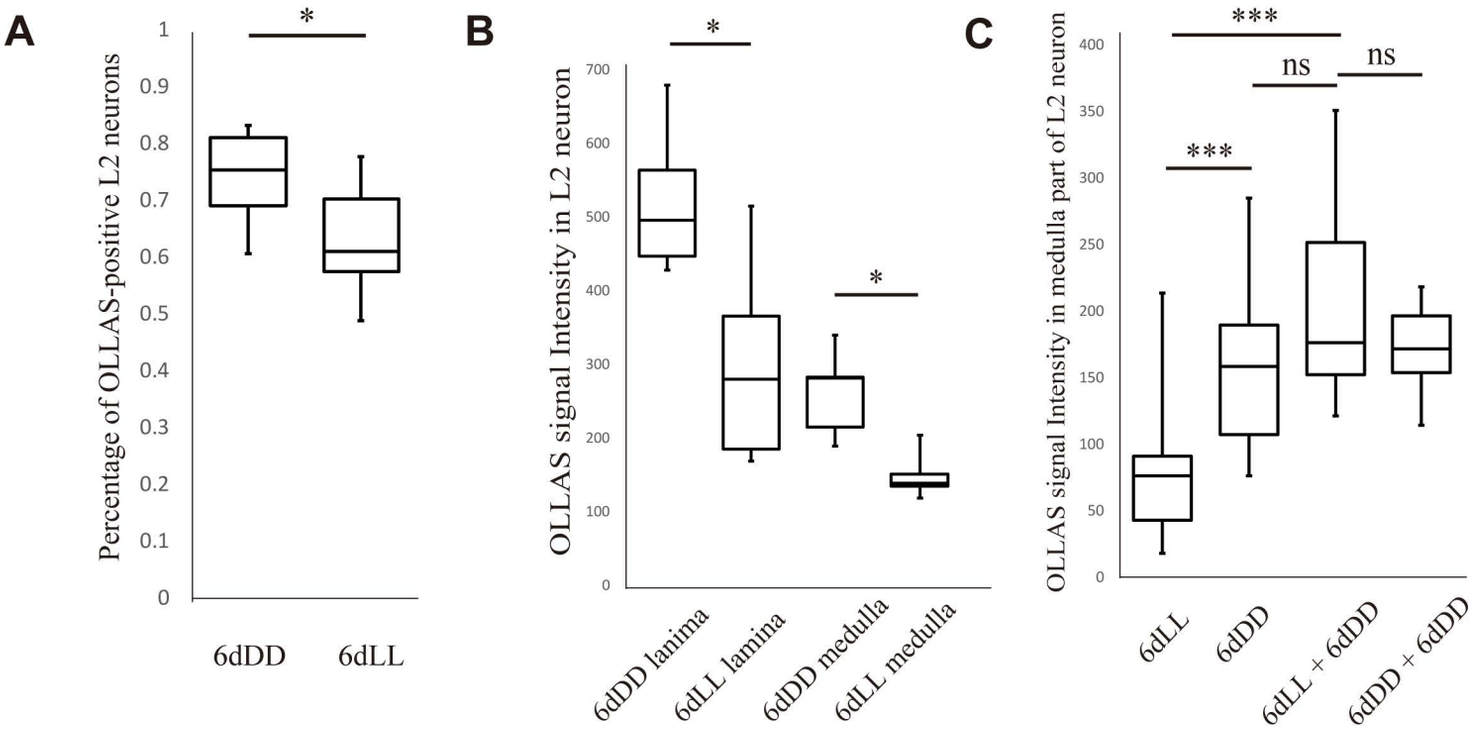


Fig.14 Pattern of HR transcriptional downregulation in L2

A. Percentage of OLLAS-positive L2 neurons within 15 μm range of depth was also decreased under 6-day LL condition (74%) compared to 6-day DD (63%) (Student' s t-test, DD: n=9; LL: n=10. p=0.0159).

B. OLLAS staining intensity change after LL/DD in lamina and medulla parts of L2 respectively.

Statistical tests between groups: p=0.001433, Kruskal-Wallis test; and

*p<0.05, post hoc Mann-Whitney U test.

C. Downregulation of histamine receptor in medulla part of L2 is reversible.

Statistical tests between groups: p=0.00001755, Kruskal-Wallis test; and

***p<0.001, post hoc Mann-Whitney U test.

Figure 15

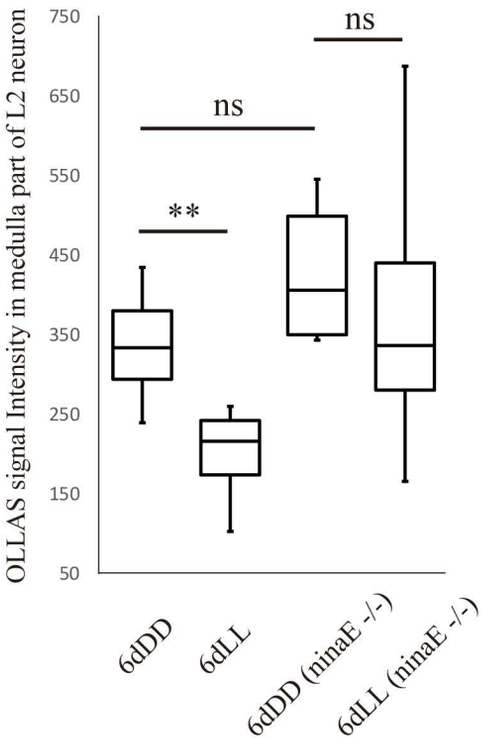


Fig.15 HR level in L2 neurons of ninaE mutant flies under various light conditions

OLLAS intensity has no significant change in L2 neurons of flies with ninaE mutant after 6-day LL.

Statistical tests between groups: p=0.005843, Kruskal-Wallis test; and **p<0.01, post hoc Mann-Whitney U test.

Figure 16

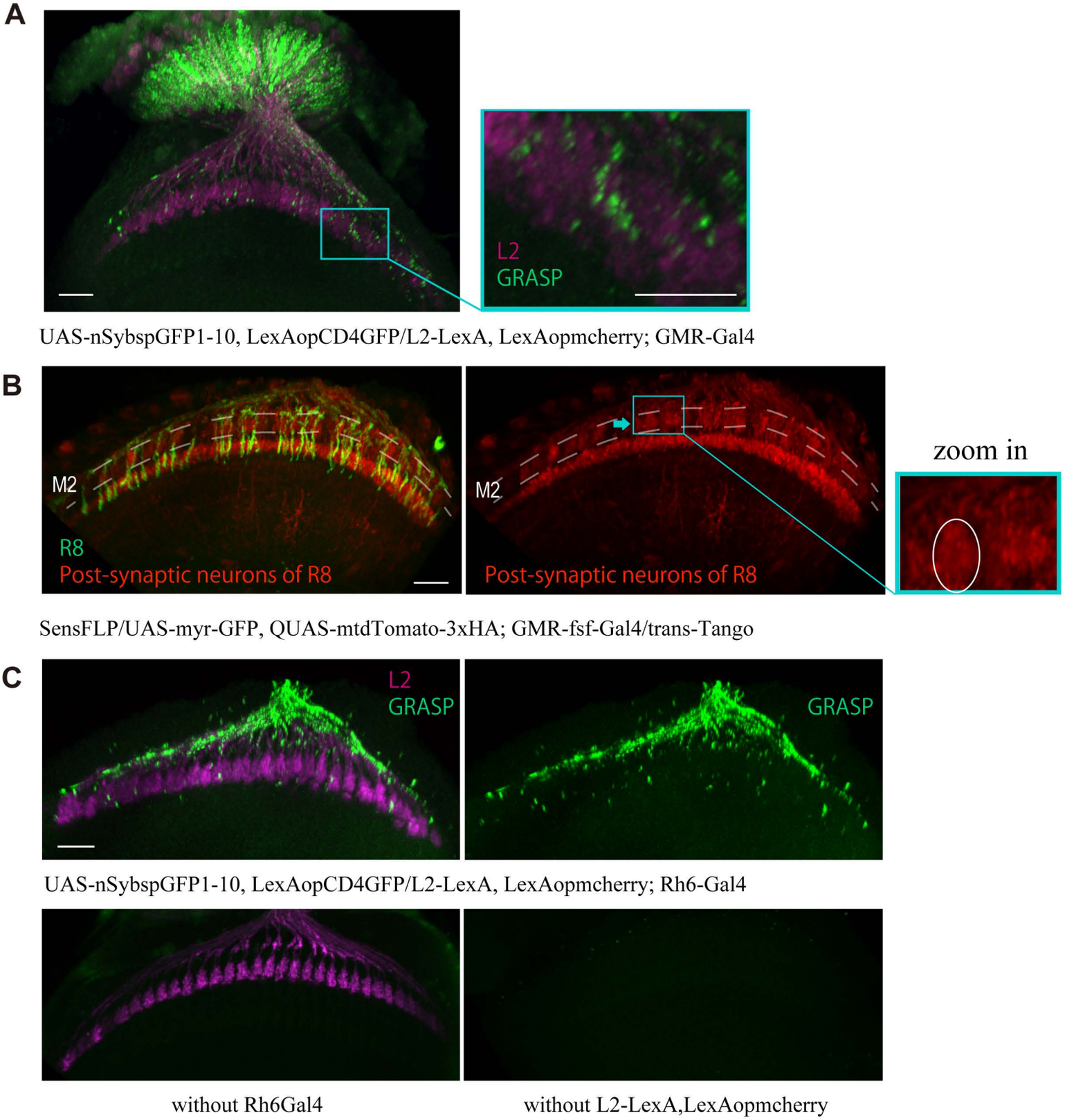


Fig.16 Connection between photoreceptor R8 and L2

A. Confirmation of the connection between photoreceptors and L2 neurons using activity-dependent GRASP showed green fluorescent signals in medulla.

Scanning was done with step size of 1 μm in a sample cross section size of 30 μm. Scale bar 10 μm.

B. Confirmation of the neurons postsynaptic to photoreceptor R8 using trans-Tango revealed L2-like neurons with oval structures. Scanning was done with step size of 1 μm in a sample cross section size of 30 μm. Scale bar 10 μm.

C. Confirmation of the connection between photoreceptor R8 and L2 neurons using activity-dependent GRASP showed green fluorescent signals in medulla.

Scanning was done with step size of 0.5 μm in a sample cross section size of 15 μm. Scale bar 10 μm.

Figure 17

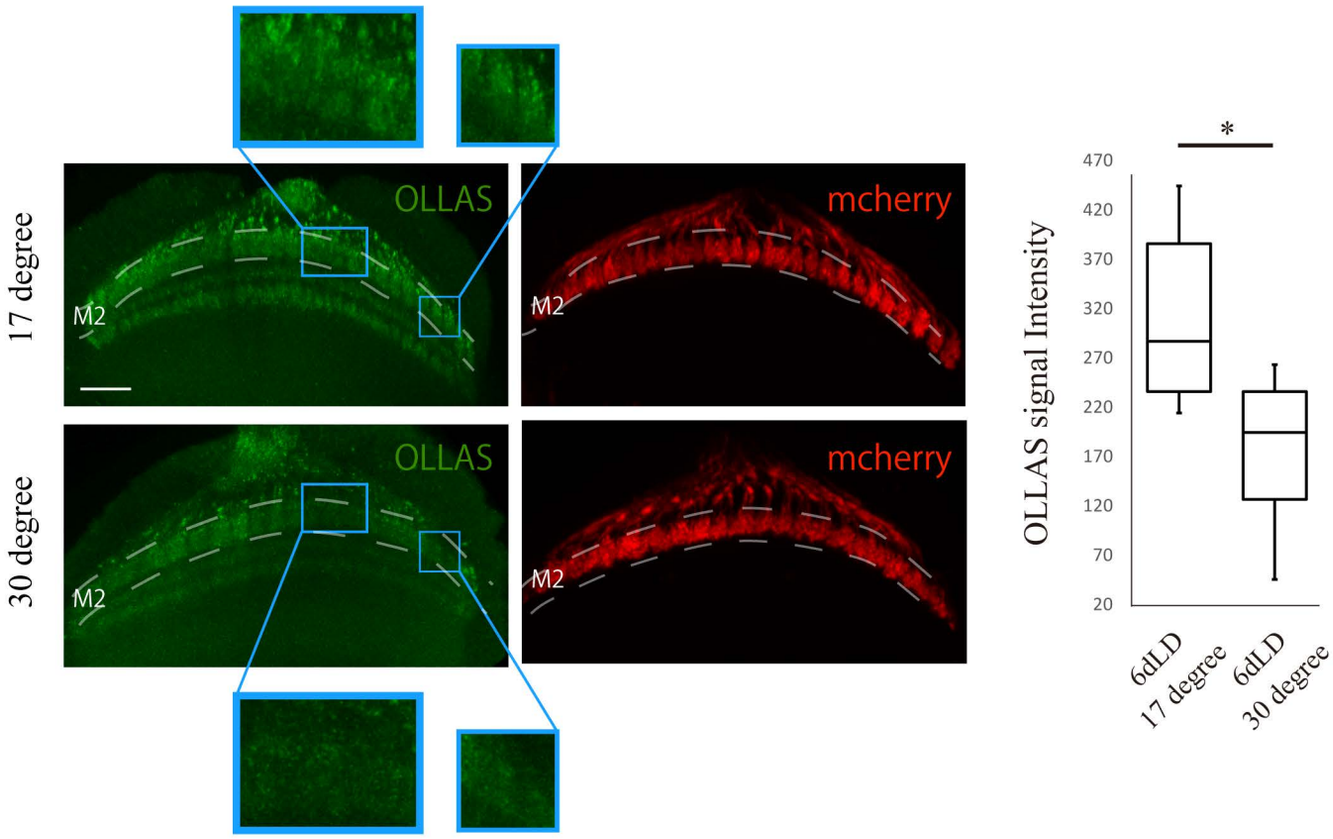


Fig.17 Effect of activating photoreceptor R8 on the HR level in L2 neurons

R8 was artificially activated using TrpA1 and histamine receptor level in the some subsets of medullar part of L2 was decreased upon R8 activation.

Scanning was done with step size of 1μm in a sample cross section size of 20μm. Scale bar 10 μm.

Student’ s t-test, 6-day LD 17°C: n=6, avg=311.76; 6-day LD 30°C: n=6, avg=175.41. *p*=0.0268.

Figure 18

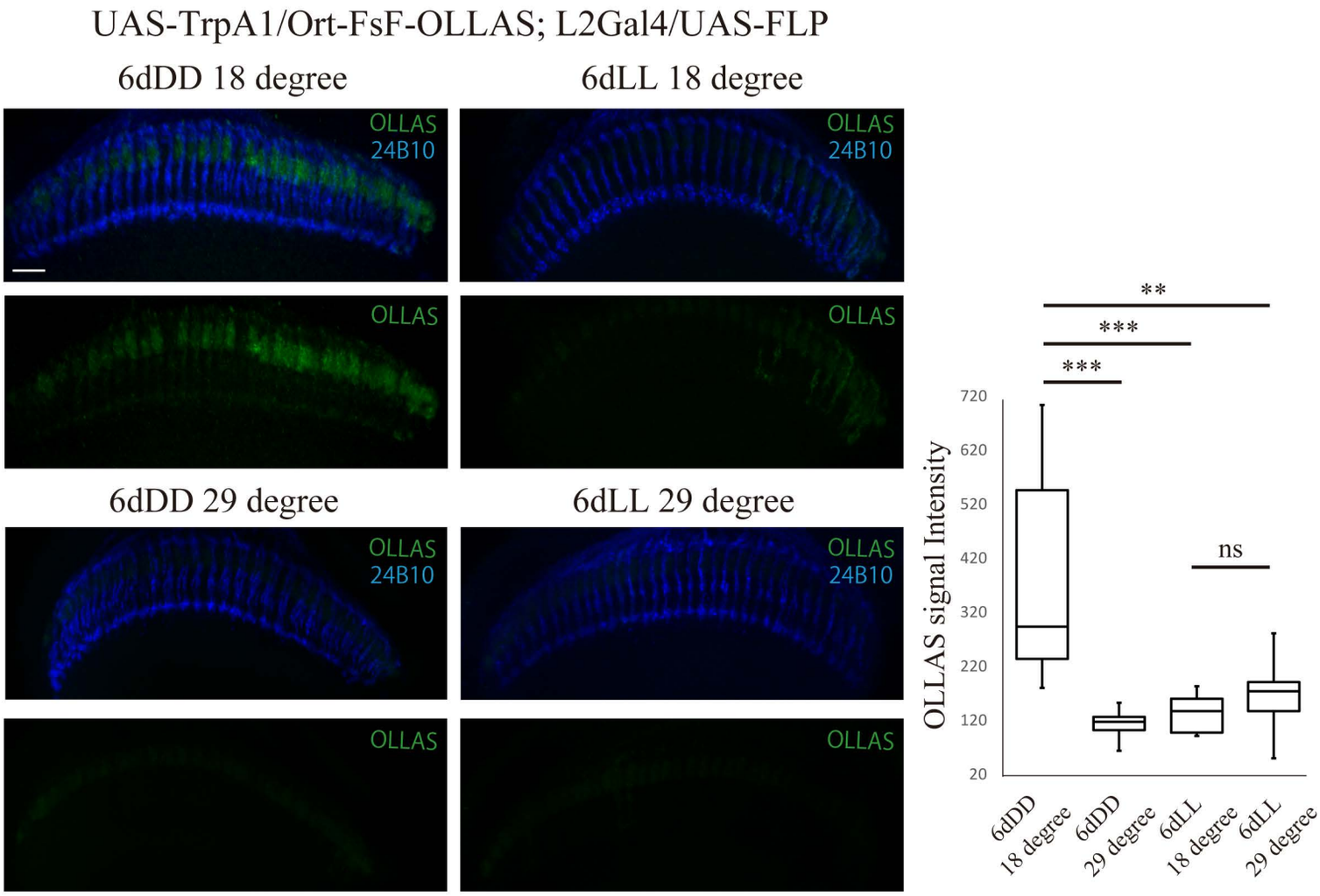


Fig.18 Histamine receptor downregulation in L2 is dependent on L2 activity

Artificial activation of L2 with TrpA1 and effect on histamine level. Scanning was done with step size of 1 μm in a sample cross section size of 15 μm. Scale bar 10 μm.

Statistical tests between groups: p=0.00002236, Kruskal-Wallis test; and

***p<0.001, **p<0.01, post hoc Mann-Whitney U test.

Figure 19

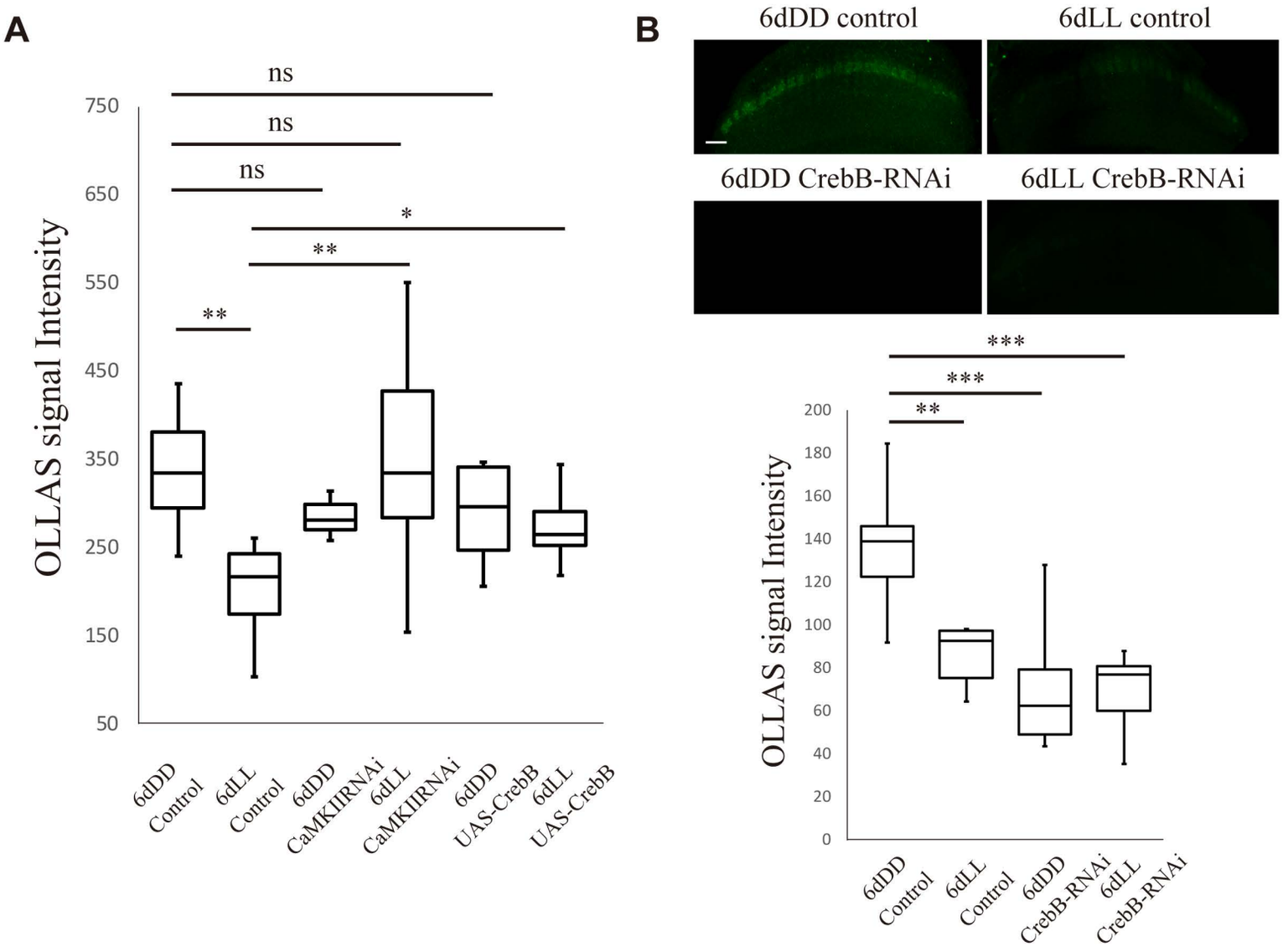


Fig.19 Regulation of histamine receptor level in L2 through CaMKII-related pathway

A. CaMKII-CrebB pathway.

Scanning was done with step size of 0.5 μm in a sample cross section size of 15 μm.

Statistical tests between groups: p=0.0151, Kruskal-Wallis test; and

**p<0.01, *p<0.05, post hoc Mann-Whitney U test.

B. Knockdown of CrebB with CrebB-RNAi largely decrease histamine receptor level in medulla part of L2.

Scanning was done with step size of 0.5 μm in a sample cross section size of 15 μm. Scale bar 10 μm.

Statistical tests between groups: p=0.000396, Kruskal-Wallis test; and

***p<0.001, **p<0.01, post hoc Mann-Whitney U test.

Figure 20

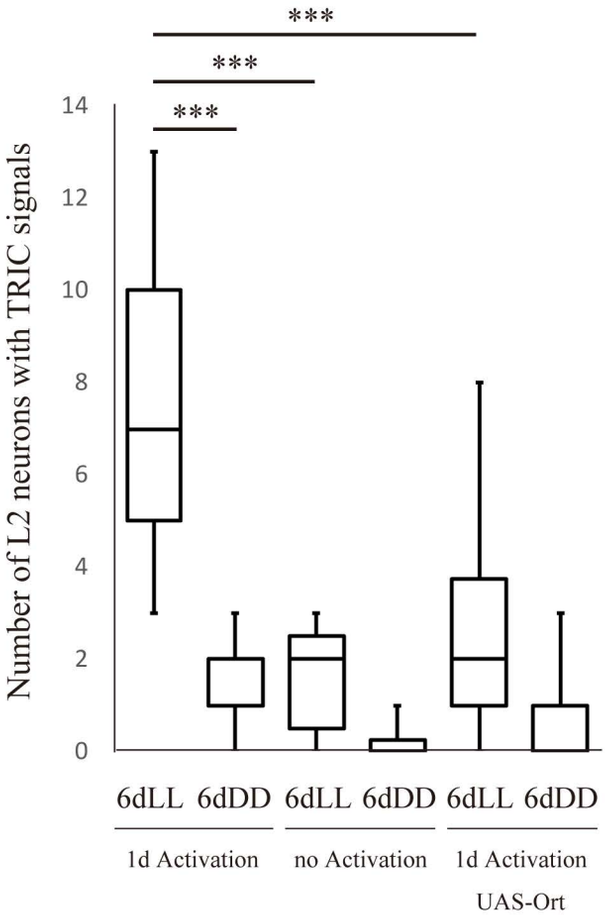


Fig.20 The change in activity responses to 1-day photoreceptor activation after prolonged light exposure derived from the Ort reduction

TRIC assays in which flies were treated by conditions as follows:

1) 6-day LL and 1-day 32 °C LL, n=11;

2) 6-day DD and 1-day 32 °C LL, n=8;

3) 6-day LL and no activation, n=7;

4) 6-day DD and no activation, n=8;

5) 6-day LL and 1-day 32 °C LL, with UAS-Ort expression, n=14;

6) 6-day DD and 1-day 32 °C LL, with UAS-Ort expression, n=13.

Statistical tests between groups: $p<0.0000001$, Kruskal-Wallis test; and ***$p<0.001$, post hoc Mann-Whitney U test.

Figure 21

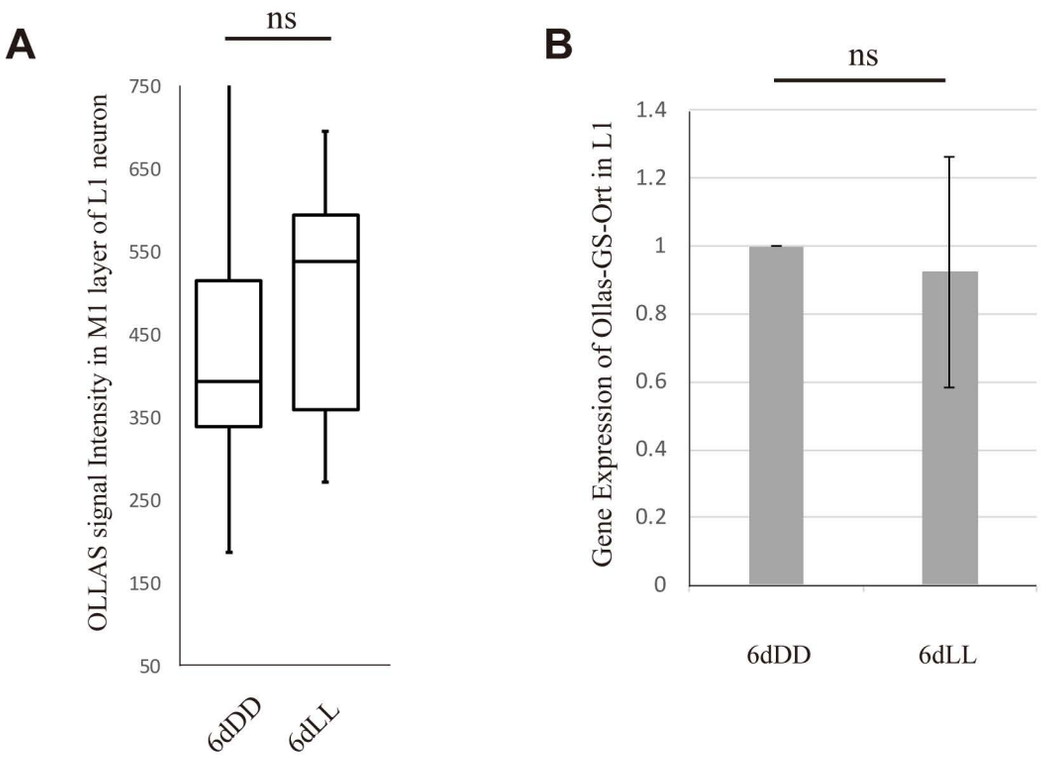


Fig.21 HR level in L1 neurons under various light conditions

A. OLLAS intensity has no significant change in L1 neurons of flies after 6-day LL compared to 6-day DD. Student’ s t-test, 6-day DD: n=12, avg=496.71; 6-day LL: n=12, avg=491.69. *p*=0.957.

B. Three independent biological replicates and several technical replicates for each biological replicates were measured for LL or DD condition. Housekeeping gene Rpl32 was used as reference.The calculation of threshold cycle (Ct) of qPCR followed 2nd Derivative Maximum method. Relative quality of LL is calculated compared to DD (defined as 1). Student’ s t-test, 6-day LL: 0.924. *p*=0.844.

Figure 22

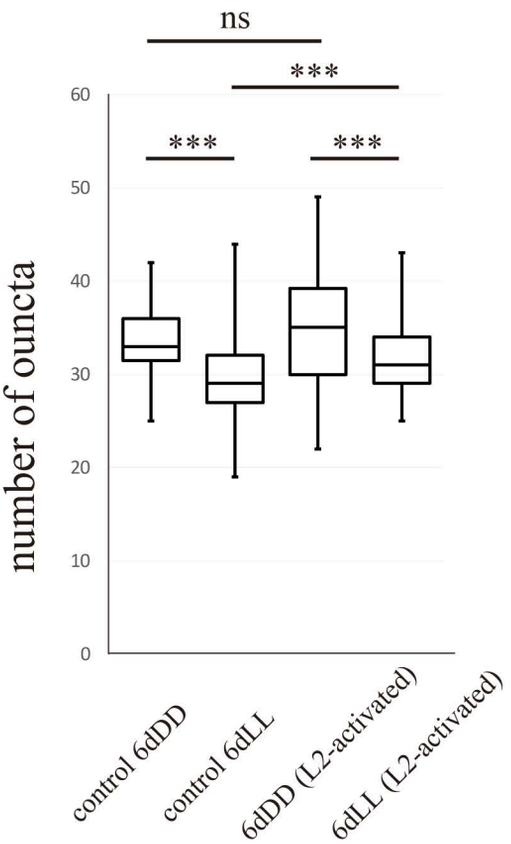


Fig.22 Activation of L2 suppresses the synaptic loss in R8

Artificially activating L2 suppresses the synaptic connection loss in R8. UAS-TrpA1 was expressed in L2 and flies were reared in 29 degree for 6 days to activate L2. Boxplot comparing the total number of Brp-GFP BAC puncta: Statistical tests between groups: $p<0.00001$, Kruskal-Wallis test; and

***$p<0.001$, post hoc Mann-Whitney U test.

Figure 23

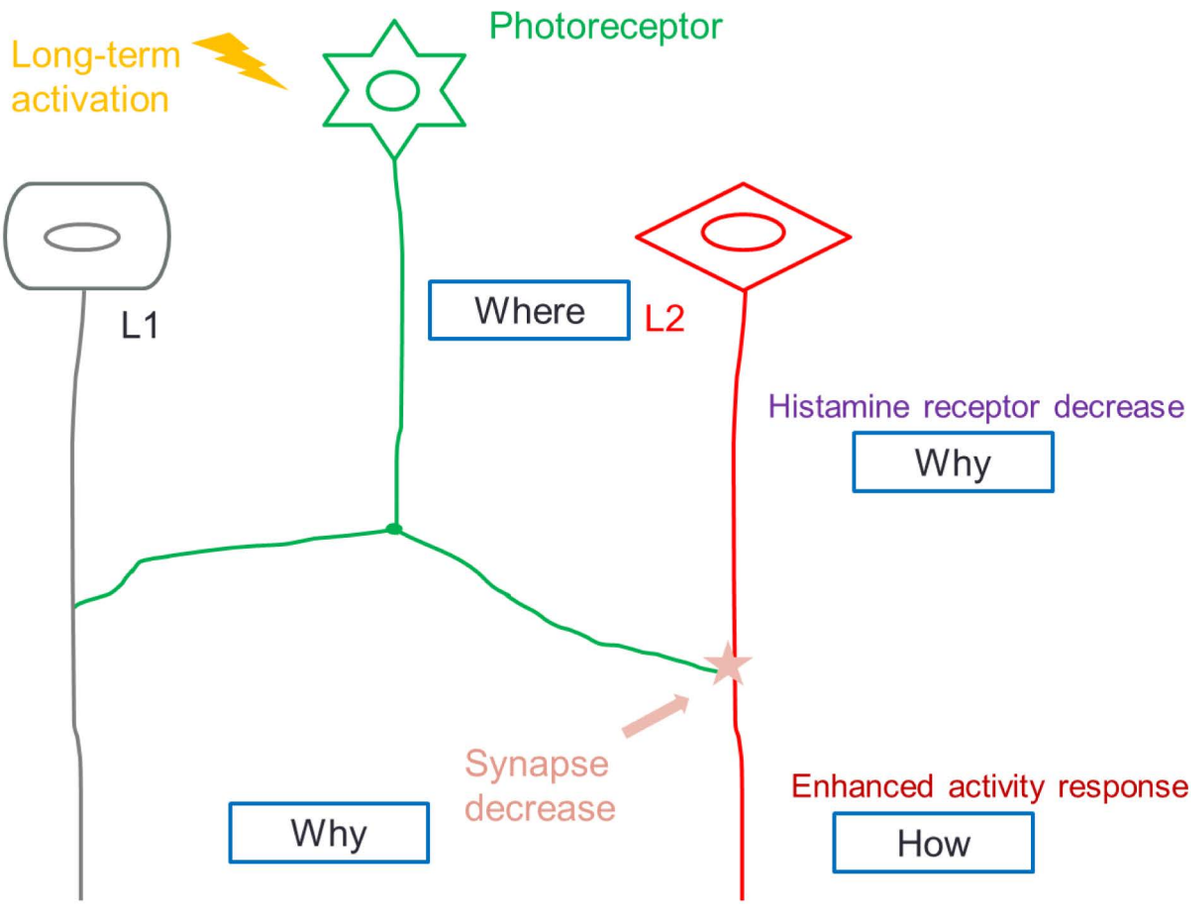


Fig.23 HR downregulation and activity response change occur specifically in L2

The activity-dependent transcriptional downregulation of the L2 HRs is partially responsible for the activity-dependent synaptic plasticity-induced circuitry response change. After the visual circuit of Drosophila receives long-term activation, synaptic plasticity involving activity-dependent transcriptional downregulation of HRs occurs specifically in L2 neurons, and then changes the activity responses of L2 upon subsequent photoreceptor activation.

Figure 24

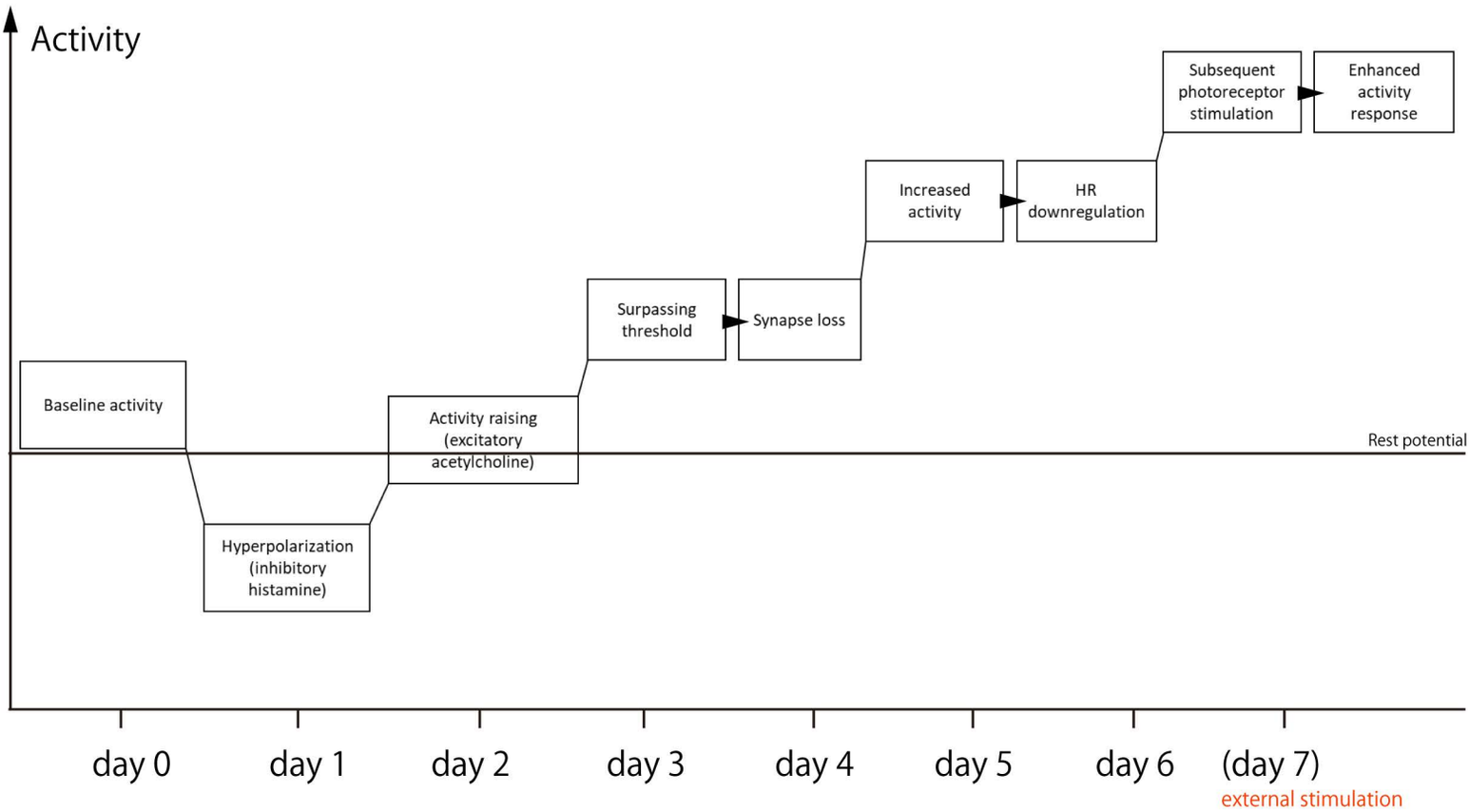


Fig.24 Time course of L2 activity in 6-day prolonged light exposure

Both R1-6 and R8 photoreceptors mainly release inhibitory neurotransmitter histamine to L2 under light condition. R8 photoreceptors also release excitatory acetyl choline to postsynaptic neurons and activate L2, which defines the baseline activity. Upon light stimulation, L2 is hyperpolarized at first but goes up back to slight depolarized state as the baseline activity after a certain period.

There may be a threshold of activity for L2 and only after 3-day LL can calcium influx surpass the threshold and trigger synapse loss, increasing L2 activity.

Further increased L2 activity downregulates histamine receptor level in L2 between day 3 to day 6.

Decreased histamine receptor level allows L2 neurons to respond more drastically to subsequent light stimuli after chronic light treatment.

Figure 25

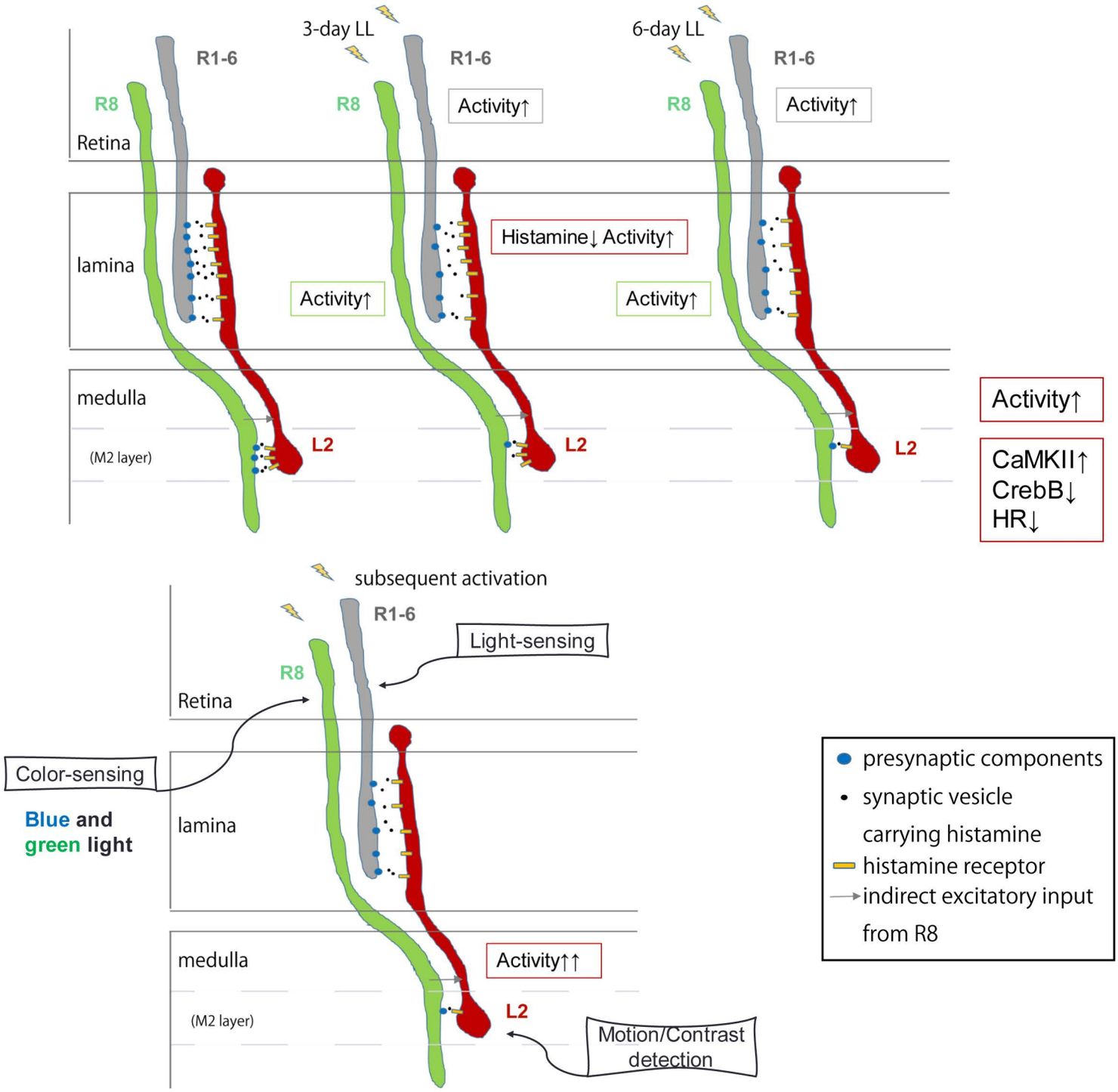


Fig.25 Model of circuitry response towards subsequent light stimuli after synaptic plasticity

R1-6 photoreceptors have connections with L2 neurons in lamina, while R8 photoreceptors have connections with L2 neurons in medulla. Both R1-6 and R8 photoreceptors mainly release inhibitory neurotransmitter histamine to L2 under light condition. R8 photoreceptors also release excitatory acetyl choline to postsynaptic neurons and activate L2 through an indirect way, which defines the baseline activity. After 3-day LL treatment, the increased calcium influx over threshold in L2 triggers reorganization of presynaptic components in AZ and synapse numbers are decreased. Photoreceptors release fewer histamine, which leads to further activation of L2 neurons. Increased activity in L2 induces downregulation of histamine receptor (HR) through CaMKII-CrebB pathway. After 6-day LL treatment, histamine receptor level is significantly decreased in L2. Downregulation of histamine receptor allows L2 neurons respond more drastically to subsequent light stimuli after chronic light treatment. This kind of regulation may potentially maintain normal function of circuitry and coordinate light-sensing, motion detection, and color vision under prolonged light exposure.

Figure 26

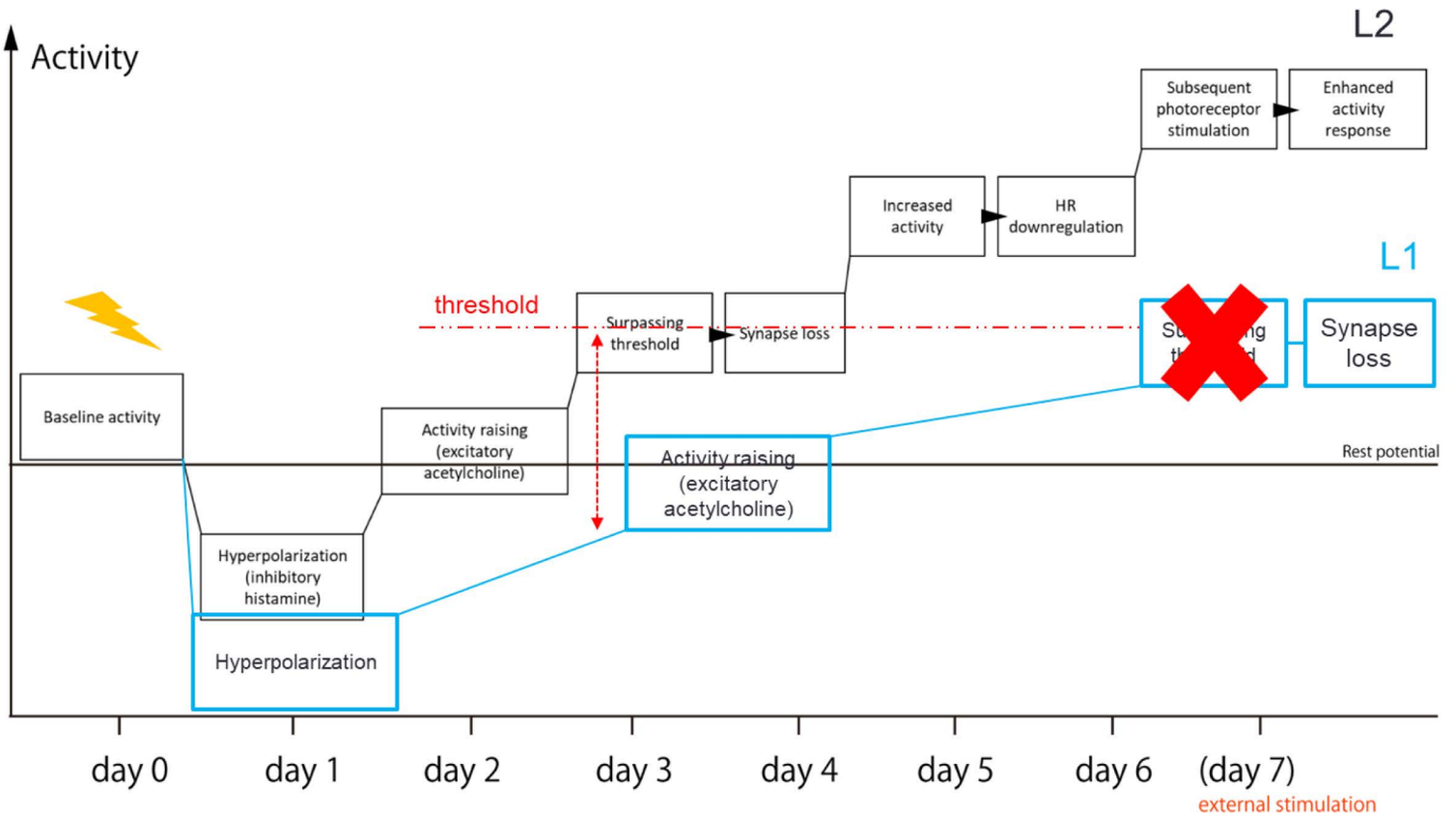


Fig.26 L1 neurons act differently from L2

Since L1 did not show HR downregulation which happened between day 3 to day 6 in L2, and no increased activity response on day 7, the branching point of L1 and L2 performances should occur before day 3. It is supposed that the response of L1 to light stimulation at very beginning may account for the phenomenon. Photoreceptors can release not only inhibitory neurotransmitter histamine but also excitatory neurotransmitter acetylcholine to the postsynaptic neurons. L2 neurons express high level of excitatory acetylcholine receptors while L1 neurons express much less, and L2 neurons also express relatively higher level of excitatory GABA-A receptors than L1.

It implies that when there is light stimulation at very beginning, L1 neurons get inhibitory input, less excitatory input and in result more intense hyperpolarization than L2 neurons and it may take much longer time for L1 neurons to go back to baseline activity. Thus, for the first 3 days L1 neurons may experience long hyperpolarization status and fail to trigger further presynaptic modification, HR regulation or activity response change like L2.