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Light-evoked synaptic activity of retinal ganglion and amacrine cells is regulated in developing mouse retina

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Abstract

Recent studies have shown a continued maturation of visual responsiveness and synaptic activity of retina after eye opening, including the size of receptive fields of retinal ganglion cells (RGCs), light-evoked synaptic output of RGCs, bipolar cell spontaneous synaptic inputs to RGCs, and the synaptic connections between RGCs and ON and OFF bipolar cells. Light deprivation retarded some of these age-dependent changes. However, many other functional and morphological features of RGCs are not sensitive to visual experience. To determine whether light-evoked synaptic responses of RGCs undergo developmental change, we directly examined the light-evoked synaptic inputs from ON and OFF synaptic pathways to RGCs in developing retinas and found that both light-evoked excitatory and inhibitory synaptic currents decreased, but not increased, with age. We also examined the light-evoked synaptic inputs from ON and OFF synaptic pathways to amacrine cells in developing retinas and found that the light-evoked synaptic input of amacrine cells is also down-regulated in developing mouse retina. Different from the developmental changes of RGC spontaneous synaptic activity, dark rearing has little effect on the developmental changes of light-evoked synaptic activity of both RGCs and amacrine cells. Therefore, we concluded that the synaptic mechanisms mediating spontaneous and light-evoked synaptic activity of RGCs and amacrine cells are likely to be different.

Keywords

Synaptic current; Patch-clamp recording; Light deprivation; Glutamatergic; GABAergic

INTRODUCTION

In the retina, many functional properties of synaptic circuitry, such as the size of receptive fields of retinal ganglion cells (RGCs), light-evoked synaptic output of RGCs, bipolar cell spontaneous synaptic inputs to RGCs, the synaptic connections between RGCs and bipolar cells, and the intrinsic excitability of RGCs, undergo continued maturation after eye opening (Bowe-Anders *et al.*, 1975; Deplano *et al.*, 2004; Giovannelli *et al.*, 2008; Qu and Myhr, 2008; Rusoff & Dubin, 1977; Tian & Copenhagen, 2001; 2003; Tootle, 1993; Wang *et al.*, 2001). In contrast, many other morphological, functional and neurochemical systems of the retina, including the number of cells and synapses, and the expression of synthesizing enzymes, transporters, receptors for neurotransmitters, have reached adult levels by the time

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of eye opening (Chan & Chiao, 2008; Elstrott *et al.*, 2008; Fisher, 1979a; Greiner & Weidman, 1981; Pow & Barnett, 2000; Redburn & Madtes, 1987, Sassoe-Pognetto & Wässle, 1997). However, little is known about the maturation of the strength of light-evoked synaptic inputs to RGCs. One goal of this study is to quantify age-dependent changes of the light-evoked synaptic inputs of RGCs from ON and OFF synaptic pathways.

Many investigators have concluded that visual deprivation has no discernible effect on retina (Baro *et al.*, 1990; Chan & Chiao, 2008; Elstrott *et al.*, 2008; Hendrickson & Booth, 1976; Kratz *et al.*, 1979; Reuter, 1976; Sherman & Stone, 1973; Wiesel & Hubel, 1963). Others have reported that visual deprivation can affect morphology and function of retinal neurons, including the number of synapses in the inner plexiform layer (IPL) (Fisher, 1979b; Sosula & Glow, 1971), the receptive field of RGCs (Di Marco *et al.*, 2009), the morphology and synaptic activity of RGCs (Giovannelli *et al.*, 2008; Tian & Copenhagen, 2001; 2003; Xu & Tian, 2007). Recent studies demonstrated that the developmental ramification of RGC dendrites into OFF lamina of IPL is selectively impaired by light deprivation in many morphological subtypes of RGCs (Xu & Tian, 2007; 2008). Genetic blockage of bipolar cell glutamate release reduces the synaptic formation of ON-OFF RGCs in ON sublamina of IPL without alteration of dendritic formation in the OFF sublamina (Kerschensteiner *et al.*, 2009). These results suggest that the synaptic transmission between ON and OFF bipolar cells and RGCs might be differentially regulated. A second goal of this study is to determine whether light deprivation affects the development of RGC ON and OFF synaptic inputs differently.

Despite the increasing evidence for the experience-dependent regulation of RGC synaptic activity, little is known about whether amacrine cell synaptic activity is regulated in development. In this study, we also examined the synaptic activity of amacrine cells in developing retinas. Accordingly, we recorded light-evoked synaptic activity of morphologically identified RGCs and displaced amacrine cells from mice raised under cyclic light/dark conditions and constant darkness. Our results demonstrated that the light-evoked synaptic activity of RGCs and displaced amacrine cells are regulated in developing mouse retina. However, light deprivation has minimum effect on the developmental changes of light-evoked synaptic activity of these cells.

EXPERIMENTAL PROCEDURES

Animals and dark rearing

Retinas were obtained from C57BL/6J mice (The Jackson Laboratory, Bar Harbor, Maine, USA). The procedures for animal preparation have been described in detail previously (Vistamehr & Tian, 2004; Xu & Tian, 2007). Briefly, the control animals were raised under 12:12 hour cyclic light/dark conditions. Dark reared animals were housed in conventional mouse cages, which were placed in a continuously ventilated light-tight box. The temperature and humidity inside the box were continuously monitored and balanced by adjusting the speed of the ventilating fan. The box was placed in a light-tight room located in the same facility as control animals. All the procedures of daily monitoring and routine maintenance of dark reared mice were conducted under infrared illumination. Animals reared under cyclic light/dark conditions were dark-adapted for at least 30 minutes before experiments. Dark reared mice were transferred from the dark room to the recording room in a light-tight transfer box. All procedures of handling, maintenance and preparation of animals met the NIH guidelines and were approved by the Animal Care and Use Committee of Yale University.

Retinal whole-mount preparation for patch-clamp recordings

The procedures of mouse retinal whole-mount preparation for patch-clamp recordings are similar to the procedures described previously (Tian & Copenhagen 2001, 2003). Briefly, the eyes were enucleated and hemisected at the ora serata under infrared illumination immediately after euthanasia. The cornea, iris, lens, and vitreous were removed from eyes immersed in a Petri dish filled with oxygenated extracellular solution, which contained (in mM) NaCl 124, KCl 2.5, CaCl₂ 2, MgCl₂ 2, NaH₂PO₄ 1.25, NaHCO₃ 26, and glucose 22 (pH 7.35 with 95% O₂ and 5% CO₂) and the retina was then detached from the eyecup. All chemicals for the solution were obtained from Sigma (St. Louis, MO, USA).

The detached retina was placed on a piece of nitrocellulose filter paper (Millipore Corp., Bedford, MA, USA) with photoreceptors facing the paper. A small, 1 mm diameter hole was cut at the center of the filter paper before the retina was placed so the cells in the center area could be visualized under infrared illumination. The mounted retina with the filter paper was placed in a recording chamber with the RGCs facing up and was held in a fixed position with a miniature manipulator. The chamber was then mounted on the stage of a Nikon Eclipse (E600FN) microscope equipped with both infrared and epifluorescent illumination so the recording electrodes and cells can be visualized under infrared illumination before electrophysiological recordings and fluorescent images of the recorded cells labeled with Lucifer Yellow (LY) can be obtained after the light response recordings. The retina was continuously perfused during the recording at 34°C.

Synaptic current recordings using patch-clamp on a retinal whole-mount preparation

The pipette solution for whole-cell patch clamp experiments contained (in mM) CsOH 120, D-gluconic acid 120, CaCl₂ 1, MgCl₂ 2, EGTA 11, HEPES 20, and glucose 30 (pH 7.2) with 0.5% Lucifer yellow. Cells were randomly selected and patched under infrared illumination. Recordings began 3 to 5 minutes after the formation of whole cell configuration. Light-evoked excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) were recorded at the holding potentials of -70 mV and 0 mV, respectively. Light-evoked excitatory postsynaptic potentials (EPSPs) were recorded at current-clamp mode without current injection. Membrane currents were amplified with an EPC 9 amplifier and data was collected using a PC-based interface (Instrutech Corporation, Great Neck, NY, USA) run by HEKA software (Pulse+PulseFit, HEKA Elektronik GmbH, Germany). The signals were filtered at 1 kHz. The light stimulation was generated with a LED emitting 567 nm light.

Imaging recordings on live and fixed retinal preparations

After physiological recordings, fluorescent images of LY labeled cells were taken at both dendritic and somatic levels before removal of the patch electrode for initial morphological identification. The images were collected using the software NeuroLucida (MicroBrightField, Inc., Williston, VT, USA). Then the retina was prepared for immunostaining of LY-labeled cells using a similar procedure described previously (Xu and Tian, 2007) for further morphological confirmation. In brief, the retina mounted on nitrocellulose filter paper was fixed with 4% paraformaldehyde in 0.01 M phosphate-buffered saline (PBS, pH 7.4) for 30 min at room temperature. Fixed retinas were washed 10 min × 3 in 0.01 M PBS. After blocked in 10% normal donkey serum, retinas were incubated with a rabbit polyclonal anti-LY antibody (1:200, Sigma-Aldrich, St. Louis, MO, USA) for 1 day at 4°C. A secondary antibody (donkey anti-rabbit antibody) conjugated with FITC (Invitrogen, Carlsbad, CA, USA) at 1:100 dilution was used to reveal the anti-LY labeling at room temperature for 1 hour.

Then the retinas were flat mounted on Super-Frost Plus slides (Fisher Scientific, Pittsburgh, PA, USA) with Vectashield (Vector Laboratories, Burlingame, CA, USA). LY-labeled RGCs and displaced amacrine cells were identified visually and the fluorescent images were collected with a dual channel Olympus FV5-PSU confocal microscope (Optical Analysis Corporation, Nashua, NH, USA) with a PlanApo X60 oil lens (numerical aperture: 1.4) for further morphological analysis based on previous classifications (Muller et al., 2007; Sun et al., 2002; Volgyi et al., 2009).

Data analysis

Off line data analysis of patch-clamp recordings was carried out on a PC computer using the software Igor (WaveMetrics, Lake Oswego, OR, USA). Off-line image data analysis was carried out using the software IPLab (Scanalytics, Inc., Fairfax, VA, USA). Statistical analysis was performed using the software StatView (Abacus Concepts, Berkeley, CA, USA).

RESULTS

To determine whether the strength of light-evoked synaptic inputs of RGCs and amacrine cells are developmentally regulated, light-evoked EPSCs and IPSCs of RGCs and displaced amacrine cells were recorded using whole-cell patch recordings on dark-adapted whole-mount retinal preparations of young and adult mice raised under cyclic light/dark conditions. Light-evoked EPSPs and action potentials were also recorded from those cells as a measure of their synaptic outputs using whole-cell patch configurations in current-clamp mode. To examine the roles of visual experience on the development of the synaptic inputs and outputs of RGCs and displaced amacrine cells, light-evoked synaptic currents and action potentials were recorded from developing retinas of mice raised in constant darkness.

For patch-clamp recordings, the cells were visualized under infrared illumination and were randomly selected. After the recording of light-evoked synaptic currents and membrane potentials, the cells were initially identified as RGCs or displaced amacrine cells based on the LY labeling. Cells with visible axons were identified as RGCs (Fig. 1A), while cells without detectable axons were classified as putative displaced amacrine cells. After the recording, the retinas with LY-labeled cells were fixed and the LY signal was enhanced by anti-LY immunostaining. The morphology of LY-labeled cells was further analyzed using a confocal microscope for further cell identification. From a total of 114 cells recorded, 49 (43%) cells were identified as RGCs and 65 (57%) cells were identified as displaced amacrine cells. These numbers are similar to the estimated populations of RGCs and displaced amacrine cells located in the ganglion cell layer of mouse retina (Jeon *et al.*, 1998). With this preparation, light-evoked synaptic activity was measured from both RGCs and displaced amacrine cells. Fig. 1B shows representative recordings of light-evoked EPSCs, IPSCs and EPSPs from a RGC (left), which responded only to the onset of light stimulation, and a displaced amacrine cell (right), which responded to both the onset and the offset of the light, of a P32 and a P33 mouse, respectively.

Light-evoked EPSCs and IPSCs of RGCs are down-regulated after eye opening in developing mouse retina

We first determined whether the strength of light-evoked EPSCs of RGCs undergoes developmental regulation. Fig. 2A shows that light-evoked synaptic activity was recorded from a large variety of morphological subtypes of RGCs (Sun et al., 2002; Volgyi et al., 2009). The strength of light-evoked EPSCs was determined based on the total charge transferred, the peak amplitude and the decay kinetics of the light-evoked EPSCs. The total charge (Q) was calculated by integrating the total current evoked by light stimulation. The

decay time constants (τ) of the ON and OFF EPSCs were determined by fitting the curves from the peak amplitude of ON EPSCs to the end of the 2-second light stimulation or the peak amplitude of OFF EPSCs to the end of the 4-second recordings to an exponential decay. The charge transferred with light-evoked EPSCs was quite variable across RGCs for both ON and OFF responses. Fig. 2B shows representative EPSCs evoked by a 2-second diffuse light stimulation recorded from two RGCs of a P14 (up) and a P32 mouse (low), respectively. The RGC from the P14 mouse had a sustained inward current during the course of 2-second light stimulation. The RGC of the P32 mouse, on the other hand, had a transient inward current responding to the onset of the light stimulation, which only lasted for less than a second. Surprisingly, we found that the strength of light-evoked EPSCs decreased with age after eye opening. The average charge transferred by the light-evoked EPSCs to the onset (ON) of the light stimulation decreased 57% from P10-15 (89.5 ± 74.6 pC, mean $\pm$ SD, $n = 15$) to P30-35 (40.5 ± 26.5 pC, $n = 14$) (Fig. 2C, $p = 0.025$, $t = -2.373$, unpaired t-test). However, the average charge transferred by OFF EPSCs at P10-15 (38.5 ± 28.5 pC, $n = 13$) were not significantly different from that at P30-35 (22 ± 20.6 pC, $n = 7$, $p = 0.159$, $t = -1.47$, unpaired t-test). The developmental decrease of the charge transferred by the light-evoked EPSCs is due to a decrease of both the peak amplitude and the decay time constant of light-evoked EPSCs. The average peak amplitudes of the light-evoked ON EPSCs decreased 59% from P10-15 (246.1 ± 155.4 pA) to P30-35 (100.8 ± 40.9 pA) (Fig. 2D, $p = 0.0022$, $t = 3.388$, unpaired t-test) whereas the average peak amplitude of the OFF EPSCs at P10-15 (116.1 ± 70.9 pA) was not significantly different from that at P30-35 (96.4 ± 101.1 pA, $p = 0.6145$, $t = 1.513$, unpaired t-test). The decay kinetics of both ON and OFF EPSCs decreased during the same developmental time period, in which the ON EPSCs seem to have more prominent changes with aging in comparison with the OFF EPSCs (Fig. 2E). The average decay time constants of ON and OFF EPSCs decreased from 236.7 ± 178.4 ms and 376.7 ± 197.4 ms at P10-15 to 105.6 ± 85.5 ms and 245 ± 155 ms at P30-35 ($p = 0.0191$ and 0.1448 , $t = 2.493$ and 1.524 , unpaired t-tests), respectively. Because the similar developmental changes were shown between ON RGCs versus the ON response of ON-OFF RGCs and the OFF RGCs versus the OFF response of ON-OFF RGCs (Table 1), the ON and OFF responses obtained from different functional types of RGCs (ON or OFF versus ON-OFF) were analyzed together.

We then determined whether the developmental down-regulation of light-evoked RGC EPSCs could be the result of an age-dependent up-regulation of synaptic inhibition by examining the strength of light-evoked IPSCs of RGCs. Similar to the light-evoked EPSCs, the strength of light-evoked IPSCs was quantified by measuring the charge transferred, peak amplitudes and decay time constants. On average, the charge transferred by ON IPSCs decreased from 143.9 ± 116.9 pC ($n = 12$) at P10-15 to 23.2 ± 34.9 pC ($n = 9$) at P30-35 (Fig. 3B, $p = 0.0181$, $t = 2.631$, unpaired t-test), while the peak amplitude and decay time constant of the ON IPSCs decreased from 200.6 ± 209.9 pA and 888.2 ± 1252.6 ms at P10-15 to 39.5 ± 19 pA and 213.2 ± 199 ms at P30-35 (Figs. 3C and 3D, $p = 0.0343$ and 0.128 , $t = 2.281$ and 1.591 , unpaired t-tests). Although the average peak amplitude of OFF IPSCs decreased from 131.3 ± 124.4 pA ($n = 8$) at P10-15 to 37.3 ± 21.7 pA ($n = 7$) at P30-35, the difference between these two groups is not statistically significant (Fig. 3C, $p = 0.0713$, $t = 1.964$, unpaired t-test). These results suggest that the developmental decrease of light-evoked EPSCs of RGCs is unlikely to be the result of a developmental increase of light-evoked synaptic inhibition.

The synaptic input/output relationship of RGCs is regulated in developing retina

We further determined how the age-dependent down-regulation of light-evoked EPSCs would impact the light-evoked synaptic output of RGCs measured as the number of light-evoked action potentials. We first determined whether all RGCs fire action potentials in

responding to diffuse light stimulation in both early and later postnatal developing retina. Fig. 4A shows an image (left) of the soma of a RGC obtained from a P14 retina, which clearly shows the axon of the cell as indicated by the arrow. The right panel of Fig. 4A shows a representative 4-second recording of the membrane potential of the same cell. It is evident that this cell fired several action potentials (as shown in the inset) at both the onset and the offset of the light stimulation. Fig. 4B shows an image of the soma and axon of a P30 RGC and three consecutive recordings of light-evoked EPSPs of the cell. This RGC only responded to the onset of the light stimulation with multiple action potentials. In our recordings, 62% of RGCs fired one or more action potentials to full-field light stimulation when prominent light-evoked EPSCs were recorded in voltage-clamp recordings. The rest 38% of the RGCs did not fire action potential to full-field light stimulation because their light-evoked EPSCs were not strong enough to depolarize the cell membrane potential to the level of more positive than -40 mV. However, these RGCs fired action potentials when they are depolarized to -40 mV by injecting positive current. Therefore, we conclude that all RGCs fire one or more action potentials when they receive enough excitatory synaptic inputs.

We then compared the number of light-evoked action potentials measured at P10-15 and P30-35 from all recorded RGCs. Our data showed that the number of light-evoked action potentials increased with age after eye opening although the strength of light-evoked EPSCs decreases with age. Figs. 4C and 4D show representative light-evoked EPSCs (upper) and EPSPs (lower) of a P13 and a P35 RGC, respectively. In voltage-clamp recordings, light stimulation evoked prominent EPSCs at both onset and offset of light on the P13 RGC but only at the onset of the light on the P35 RGC with smaller amplitude. In current-clamp recordings, the same light stimulation depolarized the membrane potential of the P13 RGC from -70 mV to -40 mV at the onset and more positive than -40 mV at the offset of the light and induced one action potential at both the onset and the offset of the light stimulation. On the other hand, similar or even smaller light-evoked EPSCs depolarized the membrane potentials of the P35 RGC from -65 mV to -40 mV and induced a high frequency burst of action potentials. On average, the number of light-evoked action potentials increased from 0.9 ± 1.6 spikes/response ($n = 27$) at P10-15 to 5.5 ± 8.3 spikes/response ($n = 20$) at P30-35 (Fig. 4E, $p = 0.0078$, $t = -2.785$, unpaired t-test).

To further characterize the synaptic input-output relationship of RGCs, we plotted the number of light-evoked action potentials as a function of the total charge transferred by the EPSCs and fitted the data into a linear regression, $Y=a+bX$ (Fig. 4F). In this function, the Y indicates the number of light-evoked action potentials and the X indicates the charge transferred by light-evoked EPSCs. The constant a indicates the intersect point of the linear fitting with the Y-axis. It could be interpreted as the theoretical number of action potentials generated with minimum EPSCs and is used to describe the “excitability” of the cells. A larger number indicates a high excitability. The inverted slope ($1/b$) of the linear fitting was used to determine the input/output gain, which measures the average amount of EPSC required for generating each action potential. A small number indicates a high input/output gain. For RGCs at P10-15 (solid lines), both the excitability and the input/output gains are relatively low (0.36 action potential and 125 pC, respectively). For the RGCs at P30-35 (dash lines), both the excitability and the input/output gain increased significantly (4.36 action potential and 33 pC, respectively). However, the correlation coefficients of the linear fitting are low for both age groups (0.3 and 0.1, respectively), indicating a high variability. Taken together, these results demonstrated that the synaptic inputs, cell excitability and the input/output gain of RGCs are regulated during postnatal development after eye opening.

Light-evoked synaptic inputs, outputs and the input/output relationship of amacrine cells are also regulated in developing mouse retina

Similar to the RGCs, we also recorded light-evoked synaptic activity from a large variety of morphological subtypes of displaced amacrine cells (Fig. 5A) (Muller et al., 2007). We found that the strengths of displaced amacrine cell light-evoked EPSCs and IPSCs decreased with age after eye opening. The average charge transferred by light-evoked EPSCs of ON and OFF responses decreased 74% and 54% from P10-15 to P30-35, respectively (Fig. 5B, $p = 0.0023$ and 0.0207 , $t = -3.262$ and 2.483 , unpaired t-tests). Consistently, the average peak amplitude and decay time constant of light-evoked ON EPSCs decreased from 375.8 ± 279.1 pA ($n = 18$) and 259.5 ± 220.6 ms at P10-15 to 177.1 ± 128.6 pA ($n = 23$) and 141.8 ± 109.1 ms at P30-35 (Figs. 5C and 5D, $p = 0.0043$ and 0.031 , $t = 3.035$ and 2.238 , unpaired t-tests). However, the average peak amplitude and decay time constant of the OFF EPSCs at P10-15 (116.5 ± 81.4 pA and 260.1 ± 139.9 ms, $n = 12$) were not statistically different from that at P30-35 (116.8 ± 95.2 pA and 204.9 ± 169.9 ms, $n = 13$, $p = 0.994$ and 0.3866 , $t = -0.0008$ and 0.883 , unpaired t-tests). As for RGCs, the ON and OFF responses obtained from different functional types of displaced amacrine cells (ON or OFF versus ON-OFF) were analyzed together because the similar developmental changes were shown between ON displaced amacrine cells versus the ON response of ON-OFF displaced amacrine cells and the OFF displaced amacrine cells versus the OFF response of ON-OFF displaced amacrine cells (Table 2).

Light-evoked ON IPSCs of displaced amacrine cells seem also to be down-regulated in developing retina with significant variation (Figs. 5E–5G). The average peak amplitude of ON IPSCs decreased from 237 ± 198 pA ($n = 12$) at P10-15 to 89.9 ± 93.2 pA ($n = 13$) at P30-35 (Fig. 5F, $p = 0.0243$, $t = 2.41$, unpaired t-test). Although the average charge transfer and decay time constant of the ON IPSCs decreased from 186.5 ± 220.9 pC and 851.8 ± 1118.9 ms at P10-15 to 55.2 ± 83.2 pC and 487.4 ± 954.3 ms at P30-35, the differences between these two groups are not statistically significant (Figs. 5E and 5G, $p = 0.0671$ and 0.3888 , $t = 1.926$ and 0.878 , unpaired t-tests). The OFF IPSCs is not different between the P10-15 and P30-35 displaced amacrine cells (Figs. 5E–5G).

In addition, we examined the synaptic input/output relationship of displaced amacrine cells by correlating the light-evoked EPSCs with the number of light-evoked action potentials in developing retina. Figs. 6A and 6B show that displaced amacrine cells in both P10-15 and P30-35 mice could fire multiple action potentials in responding to full-field light stimulation. From 15 and 20 displaced amacrine cells recorded from P10-15 and P30-35 mice, 53% (8 cells) and 90% (18 cells) of displaced amacrine cells fired action potentials. Similar to RGCs, displaced amacrine cells fired more action potentials in more mature retinas. Fig. 6C shows that a large OFF EPSC from a P10 displaced amacrine cell depolarized the membrane potential to near 0 mV without evoking action potential, while Fig. 6D shows that a smaller ON EPSC from a P34 displaced amacrine cell depolarized the membrane potential to -40 mV and evoked 7 action potentials. Similar to RGCs, the displaced amacrine cells at P10-15 fire less action potential than the displaced amacrine cells at P30-35 (Fig. 6F). On average, the number of light-evoked action potentials increased from 1.7 ± 2.2 spike/response ($n = 21$) at P10-15 to 5.8 ± 4.8 spike/response ($n = 28$) at P30-35 (Fig. 6E, $p = 0.001$, $t = -3.525$, unpaired t-test). When the number of light-evoked action potentials was plotted as a function of the total charge transferred by the EPSCs and fitted into a linear regression, it shows that the excitability of displaced amacrine cells increased from 0.8 action potential at P10-15 (solid lines) to 6.2 action potentials at P30-35 (dash lines), while the “input/output gain” decreased from 143 pC at P10-15 to -500 pC at P30-35, indicating that larger EPSCs tend to induce fewer action potentials in displaced amacrine cells. Again, the correlation coefficients of the linear fitting are low for both age groups (0.5 and 0.01, respectively).

Light deprivation has minimum effect on the developmental changes of light-evoked synaptic activity of RGCs and displaced amacrine cells

Finally, we determined whether the developmental changes of light-evoked synaptic inputs and outputs of RGCs and displaced amacrine cells require visual experience. We first examined the light-evoked EPSCs, IPSCs and action potentials of RGCs from mice raised in constant darkness from birth. We found that light deprivation from birth to P30 caused minimum effect on the total charges transferred, amplitude, decay kinetics of light-evoked EPSCs and IPSCs, and the total number of light-evoked action potentials/response (Fig. 7). Similar to the RGCs, light deprivation had no detectable effect on the light-evoked synaptic inputs and outputs of displaced amacrine cells as well (Fig. 8).

DISCUSSION

Many structural and functional properties of RGCs are developmentally regulated in an activity-dependent manner. We examined the integrated excitatory and inhibitory synaptic inputs from ON and OFF synaptic pathways of RGCs and displaced amacrine cells of developing mouse retina. In contrast to the developmental changes of spontaneous synaptic activity of mouse RGCs, light-evoked EPSCs and IPSCs, especially ON response, of both RGCs and displaced amacrine cells are down-regulated in developing mouse retina. Interestingly, the developmental down-regulation of RGC and displaced amacrine cell light-evoked EPSCs is associated with an age-dependent increase of synaptic outputs measured as the number of light-evoked action potentials. In addition, light deprivation has little effect on the developmental changes of light-evoked synaptic inputs and outputs of both RGCs and displaced amacrine cells. These results strongly suggest that spontaneous and light-evoked synaptic activities of RGCs and amacrine cells are regulated by different synaptic mechanisms.

Possible mechanisms governing developmental regulation of spontaneous and light-evoked synaptic activities of RGCs

It is surprising that the strength of light-evoked synaptic responses of RGCs decreases with age. This is reflected as an age-dependent decrease of amplitude, decay time constant and charge transferred by light-evoked ON EPSCs and IPSCs. This finding is opposite to the developmental increase of the frequency of spontaneous synaptic currents of RGCs reported previously (Giovannelli *et al.*, 2008; Tian & Copenhagen, 2001). The developmental increase of the frequency of RGC sEPSCs is attributed as a developmental increase of transmitter release from pre-synaptic bipolar cells. Consistent with this idea, the developmental increase of the frequency of RGC sEPSCs does not alter the amplitude and decay kinetics of the sEPSCs, which are commonly used to describe the response property of postsynaptic receptors (Giovannelli *et al.*, 2008; Tian & Copenhagen, 2001). The developmental decrease of light-evoked EPSCs of RGCs likely reflects a down-regulation of postsynaptic responses of RGCs. This is similar to the activity-dependent homeostatic synaptic scaling described in other parts of the CNS. The synaptic scaling is thought to compensate the change of presynaptic glutamate release by adjusting the density of postsynaptic glutamate receptors in order to stabilize the synaptic response (Davis & Goodman, 1998; Turrigiano *et al.*, 1998; Turrigiano & Nelson, 2004). However, if the developmental decrease of light-evoked ON EPSCs of RGCs was a result of a reduction in the density of postsynaptic glutamate receptors, it is unlikely due to activity-dependent AMPA receptor synaptic scaling because neither the amplitude nor the decay kinetics of sEPSCs mediated by AMPA receptors changes with age in postnatal development (Giovannelli *et al.*, 2008; Tian & Copenhagen, 2001). This notion is further supported by the recent findings that the activity-dependent AMPA receptor cycling only affects extra-synaptic, but not synaptic, AMPA receptors on the ON RGCs (Xia *et al.*, 2007).

Another possible underlying synaptic mechanism for the opposite developmental changes of spontaneous and light-evoked EPSCs of ON RGCs is that glutamate released spontaneously and evoked by light stimulation activates different subtypes of glutamate receptors, namely AMPA and NMDA receptors, on postsynaptic RGCs. Many studies showed that NMDA receptors are expressed on RGC dendrites (Fletcher *et al.*, 2000; Hartveit *et al.*, 1994; Pourcho *et al.*, 2001; Zhang & Diamond, 2006; 2009) and can be activated by evoked release of glutamate from bipolar cells (Chen & Diamond, 2002; Cohen 2000; Diamond & Copenhagen, 1993; Higgs & Lukasiewicz, 1999; Lukasiewicz & McReynolds, 1985; Mittman *et al.*, 1990; Massey & Miller, 1990; Matsui *et al.*, 1998). However, the AMPA receptors are synaptically located while the NMDA receptors are perisynaptically located at the synapses between ON bipolar cells and ON RGCs (Sagdullaev *et al.*, 2006; Zhang & Diamond, 2006). Therefore, NMDA receptors do not respond to spontaneously released glutamate but respond to the light-evoked release of glutamate from ON bipolar cells (Chen & Diamond, 2002; Matsui *et al.*, 1998; Taylor *et al.*, 1995; Sagdullaev *et al.*, 2006). On the OFF RGCs, the synaptic EPSCs are dominated by non-NMDA receptors although NMDA receptors are located both synaptically and perisynaptically (Zhang & Diamond, 2009). Therefore, with developmental increase of glutamate release from presynaptic bipolar cells, the postsynaptic RGCs could respond by reducing perisynaptically expressed NMDA receptors.

However, experimental results from previous studies seem to be controversial. First, the expression of NMDA receptors on the RGCs was reported down-regulated during postnatal development in rat retina. Guenther *et al.* (2004) showed that all RGCs expressed NMDA receptors before eye opening. The number of NMDA receptor-positive RGCs and the average amplitude of NMDA receptor-mediated currents decline after eye opening. By the age of P60, less than 50% of RGCs are NMDA receptor-positive. These results support the possibility that the age-dependent down-regulation of NMDA receptor on RGCs might be the cause of the age-dependent down-regulation of light-evoked EPSCs. In contrast, Jakobs *et al.* (2007) showed that the mRNAs of the genes encoding NMDA receptors are detected in all RGCs in adult mouse retina by single cell RT-PCR. In addition, Xue *et al.* (2002) reported that the expressions of both mRNA and protein of NR1, NR2A and NR2B increased, but not decrease, steadily during postnatal development in rat retina. Therefore, it is necessary to determine how the expression of NMDA receptors and the strength of NMDA receptor-mediated synaptic current are regulated on mouse RGCs. Second, synaptic currents mediated by NR2B-containing NMDA receptors during early postnatal ages have long decay kinetics (Flint *et al.*, 1997; Shi *et al.*, 2000; Stocca & Vicini, 1998) and the NR2A-containing NMDA receptors expressed in later postnatal development are associated with short decay times (Hestrin, 1992; Shi *et al.*, 1997; Stocca & Vicini, 1998). Therefore, NR2A-containing NMDA receptors carry less transmembrane currents. This is also consistent with the age-dependent decrease of the decay time constant of light-evoked EPSCs of RGCs. However, it was reported that the expressions of NR2B is increased, but not decreased, during the postnatal development in rat retina (Xue *et al.*, 2002). Therefore, it is also necessary to determine whether mouse RGCs express NR2A and NR2B in an age-dependent manner. Third, various subunits of the NMDA receptor and the strength of NMDA receptor-mediated currents are distributed on the ON and OFF dendrites of RGCs differently. Using immune-gold staining, Zhang and Diamond (2009) showed that NR2A-containing NMDA receptors are preferentially located at OFF synapses while NR2B-containing NMDA receptors are preferentially located at ON synapses of RGCs in rat retina. This differential distribution of NMDA receptor subunits on the ON and OFF synapses suggests that the NMDA receptor-mediated EPSCs of ON RGCs would have longer decay kinetics than OFF RGCs. However, they also found that approximately 25% of the ON RGC EPSCs is mediated by NR2B-containing NMDA receptors and 25% of ON RGC EPSCs is mediated by NR2A-containing NMDA receptor. About 50% of ON RGC EPSCs is mediated

by non-NMDA glutamate receptors. On the OFF RGCs, approximately 1/3 of the EPSCs is mediated by NR2B-containing NMDA receptors and 1/3 of the OFF EPSCs is mediated by NR2A-containing NMDA receptors. Only 1/3 of OFF RGC EPSCs is mediated by non-NMDA receptors. The different contribution of NMDA receptor-mediated current in the EPSCs of the ON and OFF RGCs might result in different decay kinetic of the ON and OFF EPSCs. Consistently, our results showed that the EPSCs of OFF responses have longer decay kinetics for both RGCs and displaced amacrine cells. Finally, several previous reports have shown that light deprivation blocks the age-dependent decline of NMDA receptors expression on RGCs (Guenther *et al.*, 2004), the levels of NR2A expression in rat retina (Xue & Cooper, 2001), and the age-dependent decline of NMDA receptor-mediated response (Carmignoto & Vicini, 1992; Fox *et al.*, 1991; Guenther *et al.*, 2004). If the NMDA receptor plays a role in the developmental changes of light-evoked ON EPSCs of RGCs, light deprivation would block the age-dependent decline of the strength of light-evoked ON EPSCs. However, our results showed that light deprivation had minimum effect on the developmental decrease of light-evoked ON EPSCs. Similarly, a recent report showed that light deprivation has no significant effect on the expression of NR2A/B in rabbit retina (Chang *et al.*, 2010). Therefore, additional experiments would be necessary to determine the role of the NMDA receptor in the developmental regulation of RGC light-evoked EPSCs.

Could presynaptic inhibition contribute to the developmental changes of RGC light-evoked EPSCs?

One might argue that presynaptic inhibition might contribute to the developmental changes of RGC light-evoked EPSCs. Presynaptic inhibition, especially GABA_A receptor-mediated synaptic inhibition, plays an important role in the regulation of glutamatergic synaptic transmission between ON bipolar cells and ON RGCs but not OFF bipolar-RGC synaptic transmission (Sagdullaev *et al.*, 2006). If the expression or activation of GABA_A receptors of ON bipolar cells increases with age, this would result in an age-dependent increase of presynaptic inhibition of ON RGCs. Consistent with this idea, the frequency of RGC spontaneous IPSCs mediated by GABA receptors significantly increase with age (Tian & Copenhagen, 2001), suggesting that spontaneous GABA release from GABAergic amacrine cells increase with age. However, a recent report showed that the frequency of spontaneous IPSCs of mouse bipolar cells is relatively unchanged with age after P12 (Schubert *et al.*, 2008), implying that spontaneous presynaptic inhibition does not attribute to the developmental change of RGC light-evoked EPSCs. It is, therefore, important to determine whether light evoked activation of GABA receptors of bipolar cells is age-dependent. In addition, light-evoked IPSCs of RGCs decrease with age after eye opening, indicating that either the light-evoked GABA release from GABAergic amacrine cells or the postsynaptic GABAergic responses of RGCs decrease with age. Previous studies demonstrated a developmental increase of GABA binding and postsynaptic GABA response on RGCs in cats (Ikeda and Robbins, 1988; 1985), disapproving a developmental decrease of postsynaptic GABAergic response. On the other hand, repetitive light stimuli induce long-term depression (LTD) of GABAergic inputs to *Xenopus* tectal neuron due to a reduction in presynaptic GABA release (Lien *et al.*, 2006), supporting the idea that light-evoked GABA release from GABAergic amacrine cells to RGCs might be down-regulated with increase of visual stimulation. However, dark rearing had no effect on the developmental change of light-evoked IPSCs seems not to support this possibility. In addition, decreased GABA release from GABAergic amacrine cells would decrease the presynaptic inhibition on bipolar cells and lead to increase of glutamate release to RGCs. This is inconsistent with the decrease of EPSCs in RGCs. Therefore, direct measurement of light-evoked GABAergic synaptic inputs of bipolar cells in developing retina would help to address this possibility.

Synaptic activity of amacrine cells are also regulated in developing retina

Although the developmental regulation of RGC synaptic activity has been extensively studied, much less is known about the development of amacrine cell synaptic activity. Our current study provided direct evidence that light-evoked excitatory and inhibitory synaptic inputs of displaced amacrine cells are also developmentally regulated in a similar manner as that of RGCs. These results suggest that the development of synaptic activity of RGCs and amacrine cells are regulated by similar mechanisms. Despite the evidence that light deprivation has no significant effect on the developmental changes of RGC and displaced amacrine cell light-evoked synaptic activity, many structural and functional properties, such as the size of receptive field, the synaptic connections with ON and OFF bipolar cells and the frequency of spontaneous synaptic inputs, of RGCs are sensitive to light deprivation (Di Marco *et al.*, 2009; Giovannelli *et al.*, 2008; Sernagor & Grzywacz, 1996; Tian & Copenhagen, 2003; Xu & Tian, 2007). In addition, light deprivation increases the density of conventional synapses in the IPL of mouse retina (Fisher, 1979b), decreases dopamine concentration of dopaminergic amacrine cells in rats and guinea pigs (Melamed *et al.*, 1986; Shelke *et al.*, 1997; Spira & Parkinson, 1991), decreases noradrenaline level in rat retina (Shelke *et al.*, 1997), decreases the population of amacrine cells expressing nicotinamide adenine dinucleotide phosphate-diaphorase in golden hamster retina (Chen *et al.*, 2006), decreases the density of GABAergic amacrine cells in mouse retina (Lee *et al.*, 2006), retarded the age-dependent changes of dendritic ramification of cholinergic amacrine cells in the IPL of mouse retina (Zhang *et al.*, 2005), and increases the density of cholinergic amacrine cells in turtle retina (Lee *et al.*, 2007). All these results strongly suggest that the structure and function of amacrine cells could be regulated by visual activity. It is, therefore, important to further examine this possibility.

The intrinsic excitability of RGCs and displaced amacrine cells increases with age

Interestingly, the developmental decrease of light-evoked EPSCs did not result in a proportional reduction of the number of light-evoked action potentials of both RGCs and displaced amacrine cells. Instead, the strength of synaptic output increases with age for both RGCs and displaced amacrine cells. These results demonstrated that, in addition to the presynaptic transmitter release and postsynaptic receptor response, the intrinsic excitability of RGCs and amacrine cells are also regulated during postnatal development. A similar age-dependent increase of RGC light-evoked action potential and developmental increase of intrinsic excitability has also been described previously in cat, ferret and mouse retina (Myhr *et al.*, 2001; Qu & Myhr, 2008; Tootle 1993; Wang *et al.*, 2001). The increased intrinsic excitability is thought to be at least partially due to a reduced threshold of firing of action potentials of RGCs (Myhr *et al.*, 2001; Qu & Myhr, 2008). Therefore, it is likely that the developmental changes of the strength of the synaptic outputs of retina are regulated by several different mechanisms. It is also interesting to note that light deprivation has no significant effect on the strength of light evoked synaptic outputs of RGCs and amacrine cells. Taken together with the fact that visual deprivation impairs many other morphological and functional properties of RGCs and amacrine cells, these results support the idea that different aspects of RGC synaptic structure and function might be regulated by different mechanisms.

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Fig. 1. Light-evoked postsynaptic currents and membrane potentials were recorded from morphologically identified RGCs and displaced amacrine cells of developing mouse retina
A: Image of a RGC from a P30 mouse labeled with LY taken after patch-clamp recording. Arrow points to the axon of the cell, which confirms the cell is a RGC. Scale bar, 10 μ m. **B:** Representative light-evoked IPSCs (upper), EPSCs (middle) and EPSPs (lower) from a RGC (left) and a displaced amacrine cell (dis-AC, right) of a P32 and a P33 mouse, respectively. The light-evoked IPSCs and EPSCs were recorded with a whole-cell configuration in voltage-clamp mode at holding potentials of 0 mV and -70 mV, respectively. The light-evoked EPSPs were recorded using the same configuration as for the synaptic current recordings in a current-clamp mode with no current injection. The bars at the bottom of the traces indicate a 2-second diffuse light stimulation. All representative traces of EPSCs and

IPSC shown in this figure and the following figures were averaged from two to four recordings.

Fig. 2. The strength of light-evoked EPSCs of RGCs decreases with age

Light-evoked EPSCs were recorded from morphologically identified RGCs of mice at P10-15 and P30-35, respectively. The strength of light-evoked EPSCs of RGCs was determined based on the total charge transferred, the peak amplitude and the decay kinetics. After physiological recordings, fluorescent images of LY labeled cells were enhanced by immunostaining using an anti-LY antibody and collected using a confocal microscope for further morphological confirmation. **A:** Stacked confocal images of recorded cells representing several morphological subtypes of RGCs (Volgyi et al., 2009). The arrows indicate axons of the RGCs. **B:** Representative light-evoked EPSCs from two ON RGCs of a P14 (upper) and a P32 (lower) mouse. The dash lines overlapped with the EPSCs indicate the exponential fittings. **C:** Average total charge transferred by light-evoked EPSCs responding to the onset (ON) and the offset (OFF) of the 2-second diffuse light stimulation of RGCs from mice at P10-15 and P30-35, respectively. **D:** Average peak amplitude of light-evoked EPSCs of the ON and the OFF responses from the same two groups of RGCs as shown in panel C. **E:** Average decay time constant (DTC) of light-evoked EPSCs of the ON and OFF responses from the same two groups of RGCs as shown in panel C. Error bars

in panels C, D and E in this figure and following figures indicate standard error (SE).
Number in each bar indicates the number of cells for each group. * indicates $0.05 > p > 0.01$.
** indicates $0.01 > p > 0.001$. *** indicates $p < 0.001$.

Fig. 3. The strength of light-evoked IPSCs of RGCs decreases with age

The strength of light-evoked IPSCs of RGCs was determined based on the total charge transferred, the peak amplitude and the decay kinetics. Light-evoked IPSCs were recorded from the same two age groups of RGCs as that for light-evoked EPSCs in Fig. 2. **A:** Representative light-evoked IPSCs from RGCs of a P11 (upper) and a P31 (lower) mouse. **B:** Average total charge transferred by light-evoked ON and OFF IPSCs from mice at P10-15 and P30-35, respectively. **C:** Average peak amplitude of light-evoked ON and OFF IPSCs of the same two groups of RGCs as shown in panel B. **D:** Average DTC of light-evoked ON and OFF IPSCs of the same two groups of RGCs.

Fig. 4. The inputs/outputs relationship of RGCs was regulated in developing retina

Light-evoked action potentials were recorded from RGCs and the relationship between excitatory synaptic inputs and outputs was examined. **A:** Fluorescent image (left) focused at the soma of a cell from a P14 retina shows an identifiable axon as indicated by the arrow. The light-evoked EPSPs (right) show that this cell responded to both ON and OFF of the light stimulation. Inset shows the OFF response indicated by the dash-line box with extended time scale. The cell fired three action potentials to the offset of the light stimulation. The bar at the bottom of the trace indicates a 2-second light stimulation. **B:** Fluorescent image and three consecutive responses to the same light stimulation of an ON RGC from a P30 retina. Inset shows the ON response indicated by the dash-line box with extended time scale. Scale bars in panels A and B, 10 μ m. **C:** Representative light-evoked EPSCs (upper) and EPSPs (lower) from a P13 RGC. **D:** Representative light-evoked EPSCs and EPSPs from a P35 RGC. Inset shows the light-evoked action potentials indicated by the dash-line box with extended time scale. **E:** Average number of action potentials/response of P10-15 and P30-35 RGCs. The number in each bar indicates the number of responses, including both ON and OFF responses. **F:** Scatter plots of the number of action potentials/response as a function of the strength of light-evoked EPSCs of P10-15 and P30-35 RGCs. The strength of light-evoked EPSCs was expressed as the total charge transferred by the

EPSCs. The solid and dash lines indicate linear fits of the number of action potentials as a function of the total charge transferred by the EPSCs of P10-15 and P30-35 RGCs.

Fig. 5. The strengths of light-evoked EPSCs and IPSCs of displaced amacrine cells decrease with age

Light-evoked EPSCs and IPSCs were recorded from displaced amacrine cells of mice at P10-15 and P30-35, respectively. As for RGCs, the fluorescent images of LY labeled cells were enhanced by immunostaining using an anti-LY antibody and collected using a confocal microscope for further morphological confirmation after physiological recordings. **A:** Stacked confocal images of recorded cells representing several morphological subtypes of displaced amacrine cells (Muller et al., 2007). “*” indicates where the somas were located before the recording electrodes were removed. **B:** Average total charge transferred by light-evoked ON and OFF EPSCs of displaced amacrine cells from mice at P10-15 and P30-35, respectively. **C:** Average peak amplitude of light-evoked ON and OFF EPSCs from the same two groups of displaced amacrine cells as shown in panel B. **D:** Average DTC of light-evoked EPSCs of the ON and OFF responses of the same two groups of displaced amacrine cells. **E:** Average total charge transferred by light-evoked ON and OFF IPSCs of the same two groups of displaced amacrine cells. **F:** Average peak amplitude of light-evoked ON and OFF IPSCs of the same two groups of displaced amacrine cells. **G:** Average DTC of light-evoked ON and OFF IPSCs of the same two groups of displaced amacrine cells.

Fig. 6. The inputs/outputs relationships of displaced amacrine cells are regulated in developing retina

Light-evoked action potentials recorded from displaced amacrine cells were examined. **A:** Fluorescent image (left) focused at the soma of a cell from a P14 retina shows no identifiable axon in this cell. The light-evoked EPSPs (right) show that this cell responded to the onset of the light stimulation with a sustained depolarization. Inset shows the ON response indicated by the dash-line box with extended time scale. **B:** Fluorescent image and three consecutive light responses of an OFF displaced amacrine cell from a P30 retina. Scale bars in panels A and B, 10 μ m. **C:** Representative light-evoked EPSCs (upper) and EPSPs (lower) from a P10 displaced amacrine cell. **D:** Representative light-evoked EPSCs and EPSPs from a P34 displaced amacrine cell. Inset shows the light-evoked action potentials indicated by the dash-line box with extended time scale. **E:** Average number of action

potentials/response of P10-15 and P30-35 displaced amacrine cells. The number in each bar indicates the number of responses, including both ON and OFF responses. **F:** Scatter plots of the number of action potentials/response as a function of the total charge transferred by the light-evoked EPSCs of P10-15 and P30-35 displaced amacrine cells. The solid and dash lines indicate linear fits of the number of action potentials as a function of the total charge transferred by the EPSCs of P10-15 and P30-35 displaced amacrine cells.

Fig. 7. Light deprivation has minimum effect on the light-evoked synaptic activity of RGCs
 Light-evoked EPSCs, IPSCs and action potentials were recorded from RGCs of mice raised in constant darkness. **A:** Average total charge transferred by light-evoked ON and OFF EPSCs of RGCs from mice raised under cyclic light (P30-35L) and constant darkness (P30-35D), respectively. **B:** Average total charge transferred by light-evoked ON and OFF IPSCs of RGCs from mice raised under cyclic light (P30-35L) and constant darkness (P30-35D), respectively. **C:** Average peak amplitude of light-evoked EPSCs of the ON and the OFF responses from the same two groups of RGCs as shown in panel A. **D:** Average peak amplitude of light-evoked IPSCs of the ON and the OFF responses from the same two groups of RGCs as shown in panel B. **E:** Average DTC of light-evoked EPSCs of the ON and OFF responses from the same two groups of RGCs as shown in panel A. **F:** Average DTC of light-evoked IPSCs of the ON and OFF responses from the same two groups of RGCs as shown in panel B. **G:** Average number of action potentials/response of RGCs of mice raised under cyclic light and constant darkness. **H:** Scatter plots and linear fits of the number of action potentials/response as a function of the total charge transferred by the light-evoked EPSCs of RGCs from mice raised under cyclic light (P30-35L) and constant

darkness (P30-35D), respectively. In panels A and B, the number in each bar indicates the number of cells.

Fig. 8. Light deprivation has no significant effect on the light-evoked synaptic activity of displaced amacrine cells

Light-evoked EPSCs, IPSCs and action potentials were recorded from displaced amacrine cells of mice raised in constant darkness. **A:** Average total charge transferred by light-evoked ON and OFF EPSCs of displaced amacrine cells from mice raised under cyclic light (P30-35L) and constant darkness (P30-35D), respectively. **B:** Average total charge transferred by light-evoked ON and OFF IPSCs of displaced amacrine cells from mice raised under cyclic light (P30-35L) and constant darkness (P30-35D), respectively. **C:** Average peak amplitude of light-evoked EPSCs of the ON and the OFF responses from the same two groups of displaced amacrine cells as shown in panel A. **D:** Average peak amplitude of light-evoked IPSCs of the ON and the OFF responses from the same two groups of displaced amacrine cells as shown in panel B. **E:** Average DTC of light-evoked EPSCs of the ON and OFF responses from the same two groups of displaced amacrine cells as shown in panel A. **F:** Average DTC of light-evoked IPSCs of the ON and OFF responses from the same two groups of displaced amacrine cells as shown in panel B. **G:** Average number of action potentials/response of RGCs of mice raised under cyclic light and constant darkness. **H:** Scatter plots and linear fits of the number of action potentials/response as a function of the total charge transferred by the light-evoked EPSCs of displaced amacrine cells from mice raised under cyclic light (P30-35L) and constant darkness (P30-35D), respectively. In panels A and B, the number in each bar indicates the number of cells.

Table 1

RGC light-evoked EPSCs and IPSCs of developing and light deprived mouse retina.

	Age	ON of ON cells	ON of ON-OFF cells	OFF of OFF cells	OFF of ON-OFF cells
EPSC Amp	P10-14	309.11±66.92 (n=4)	243.9±48.92 (n=10)	137.24±14.74 (n=2)	120±24, 1 (n=10)
	P30-34L	117.82±9.95 (n=10)	58.24±15 (n=4)	194.89±117.39 (n=2)	63.19±21.95 (n=4)
	P30-34D	238.86±41.83 (n=5)	124.69±21.8 (n=8)	191.68±38.77 (n=4)	115.48±23.2 (n=8)
EPSC DTC	P10-14	256.09±94.36	215.04±59	485.04±278.55	360.42±56.11
	P30-34L	118.02±29.11	74.58±33.48	179.39±38.51	274.31±103.61
	P30-34D	151.47±35.43	297.53±54.06	283.21±74.6	269.2±41.34
EPSC Q	P10-14	-193.52±27.71	-53±10.3	-64.69±4.31	-34.1±8.24
	P30-34L	-44.99±8.73	-29.28±11.43	-38.34±15.94	-15.86±10.36
	P30-34D	-60.1±16.17	-39.37±9.73	-29.17±14.72	-20.37±3.68
IPSC Amp	P10-14	351.8±148.29 (n=3)	141.16±77.25 (n=7)	65.31 (n=1)	163.42±86.33 (n=4)
	P30-34L	41.78±10.21 (n=5)	43.75±6.25 (n=2)	31.3±7.55 (n=2)	40.66±20.84 (n=3)
	P30-34D	42.29±21.02 (n=3)	26.05±9.17 (n=6)	33.48±6.4 (n=4)	75.38±61.2 (n=3)
IPSC DTC	P10-14	1740.29±872.92	198.78±72.21	196.37	141.99±34.6
	P30-34L	208.7±109.85	89.163±5.52	135.3±72.57	359.82±124.38
	P30-34D	188.11±23.58	649.22±286.83	326.83±92.39	293.81±95.47
IPSC Q	P10-14	255.35±49.67	78.84±39.66	53.13	33.16±14.1
	P30-34L	10.68±4.8	56.83±44.6	2.31±0.73	56.88±36.65
	P30-34D	14.68±1.69	11.71±3.09	17.08±8	37.05±26.52
AP	P10-14	1.43±0.8 (n=4)	1.07±0.68	0.33±0.33	0.77±0.43
	P30-34L	3.61±1.99 (n=10)	3.27±1.46	23.5±9.5	3.35±1.76
	P30-34D	3.93±3.37 (n=5)	5.37±1.51	1.17±1.17	3.4±1.35

Table 2
Displaced AC light-evoked EPSCs and IPSCs of developing and light deprived mouse retina.

	Age	ON of ON cells	ON of ON-OFF cells	OFF of OFF cells	OFF of ON-OFF cells
EPSC Amp	P10-14	403.15±91.79 (n=11)	332.92±94.72 (n=7)	54.38 (n=1)	157.27±31.82 (n=7)
	P30-34L	201±36.29 (n=15)	132.31±33.47 (n=8)	223.37±95.76 (n=3)	94.62±10.03 (n=8)
	P30-34D	209.93±59.33 (n=12)	125.05±23.59 (n=4)	94.68±27.24 (n=4)	65.2±17.41 (n=8)
EPSC DTC	P10-14	298.29±79.51	198.48±46.16	435.79	235.25±44.97
	P30-34L	124.55±28.99	174.06±36.03	214.16±116.43	181.39±45.42
	P30-34D	186.71±46.52	94.26±44.2	176.2434±33.95737	184.08±28.73
EPSC Q	P10-14	-213.32±63.7	-97.44±21.34	-50.48	-58.24±12.1
	P30-34L	-38.87±6.74	-54.03±18.89	-24.21±4.59	-20.14±5.68
	P30-34D	-94.06±16.1	-14.89±5.84	-17.81±9.27	-22.77±5.98
IPSC Amp	P10-14	229.23±66.28 (n=8)	252.41±123.45 (n=4)		126.59±41.04 (n=4)
	P30-34L	110.346±40.57 (n=8)	54.12±16.11 (n=4)		50.31±19.69 (n=3)
	P30-34D	131.06±42.13 (n=5)	70.26±43.15 (n=3)		94.91±58.16 (n=3)
IPSC DTC	P10-14	1074.98±468	405.32±160.8		166.43±35.6
	P30-34L	652.97±429.91	219.37±24.63		134.79±35.82
	P30-34D	311.27±134.82	94.81±13.72		175.99±52.36
IPSC Q	P10-14	191.03±76.28	177.43±132.45		15.62±5.2
	P30-34L	70.19±35.4	22.25±9.52		9.95±2.15
	P30-34D	47.89±22.75	7.09±3.36		5.89±5.22
AP	P10-14	2.62±1.09	0.75±0.36	2.25	1.53±1.06
	P30-34L	5.19±1.22	6.52±2.42	10.66667	5.71±1.85
	P30-34D	2.56±0.88	4.75	8.333333±6.202318	7.67±4.85