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Visual experience and maturation of retinal synaptic pathways ☆

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Abstract

The retinal synaptic network continues its maturational refinement after eye opening in mammals. This synaptic refinement is reflected in changes of retinal neuron synaptic activity and connectivity. In mature retina, the dendrites of retinal ganglion cells (RGCs) in the inner plexiform layer (IPL) of retina are separated into ON or OFF sublamina. At early developmental stage, however, the dendrites of most RGCs are ramified throughout the IPL. Recently we found that the postnatal maturational processes converting bistratified ON–OFF responsive RGCs to monostratified ON and OFF responsive RGCs depend upon visual stimulation after eye opening.

Keywords: Synaptic plasticity; Synaptic pathway; Visual deprivation; Retinal ganglion cells

1. Introduction

The neuronal image of the visual scene that is processed by the retina is conducted to the brain by a set of separated spatio-temporal pathways. These parallel pathways are formed with different cellular components and synaptic structures in retina. One of the fundamental features of these parallel signal processing is the functional separation of the increment and decrement of luminance signals into ON and OFF pathways (Hartline, 1938). The separation of these two pathways starts at bipolar cell level in cone-driven system and amacrine cell level in rod-driven system in mammalian retinas. They remain separated to a large extent in the retinal ganglion cell (RGC) level, the lateral geniculate nucleus (LGN) and the visual cortex. This separation of ON and OFF pathways forms the basis of virtually all visual signal processing in higher centers of visual system.

RGCs, the output neurons of the retina, transfer specific aspects of visual signals from retina to the higher levels of visual system. The final pattern of visual scene conducted to the brain by each RGC is largely determined by its synaptic inputs from bipolar and amacrine cells. There are about 11 morphologically distinctive subtypes of RGCs in adult mammalian retina (Rockhill, Daly, MacNeil, Brown, & Masland, 2002). These cells exhibit strikingly varied dendritic architecture (Cajal, 1893; Rodieck, 1998; Wässle & Boycott, 1991), axonal projection patterns (Garraghty & Sur, 1993; Tootle & Friedlander, 1989; Yamagata & Sanes, 1995a, 1995b) and responses to light stimulus. To obtain and maintain the functional specificity of each RGC, it is crucial that RGCs only form synapses with appropriate presynaptic bipolar and amacrine cells. RGCs form their synaptic connections by ramifying their dendrites into distinct sublaminae of the inner plexiform layer (IPL) of retina and, therefore, only contact certain types of bipolar and amacrine cells. Much has been learned about the functional and morphological specificity of RGCs in

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adult retina. However, much less is known about how each individual RGC forms its functionally and morphologically specific synaptic connections with presynaptic cells during development. Recent studies showed that the development of RGC dendritic ramification and synaptic connections undergoes a dramatic activity-dependent remodeling (Wong & Ghosh, 2002).

Numerous studies have shown that visual experience is necessary for the normal development of the visual cortex. Visual deprivation dramatically alters the synaptic connections and function of visual cortex (Carmignoto & Vicini, 1992; Cynader & Mitchell, 1980; Fox, Daw, Sato, & Czepita, 1991; Gordon & Stryker, 1996; Kirkwood, Rioult, & Bear, 1996; Mower, 1991). In dark reared animals, visual cortical cells show rapid habituation to repeated stimulation, decreased orientation and directional selectivity, enlarged receptive fields, and impaired spatial resolution and response. The formation of normal binocular connections within the visual cortex is also delayed by rearing animals in darkness. These modifications have served as a paradigmatic model of activity-dependent plasticity in the nervous system. Plasticity in pre-cortical regions of the visual system has recently been reported. Dark reared ferrets have a higher percentage of ON–OFF cells in the dorsal lateral geniculate nucleus (dLGN), the principal target of RGC axons, than animals raised in cyclic light (Akerman, Smyth, & Thompson, 2002). This result of the visual experience-dependent regulation of ON–OFF responsive neurons in developing dLGN is consistent to a recent study on the experience-dependent maturation of ON–OFF pathways in mouse retina (Tian & Copenhagen, 2003). Here I will focus on the recent advances in our understanding of how visually evoked activity modulate the formation of retinal synaptic pathways, particularly ON and OFF pathways, at RGC level.

2. Cellular structure of retinal ON and OFF pathways

The separation of ON and OFF pathways starts at the first synapse of the retina (Fig. 1). In vertebrate retina, light stimulation hyperpolarizes the membrane potentials of rods and cones and decreases the synaptic release of glutamate from these cells. Glutamate released from photoreceptors activates an ionotropic glutamate receptor on cone-driven OFF bipolar cells and depolarizes their membrane potentials. On the cone-driven ON bipolar and rod-driven bipolar cells, glutamate activates a 2-amino-4-phosphonobutyrate (APB) sensitive metabotropic glutamate receptor (Shiells, Falk, & Naghshineh, 1981; Slaughter & Miller, 1981) and hyperpolarizes the membrane potentials of these cells through a G-protein coupled mechanism (Nawy & Jahr, 1990; Shiells & Falk, 1990). This sign reversed action of glutamate on the ON and OFF bipolar cells separates the increment and decrement luminance signals into ON and OFF pathways.

The separation of ON/OFF pathways in RGCs relies on the synaptic connections between bipolar and RGCs

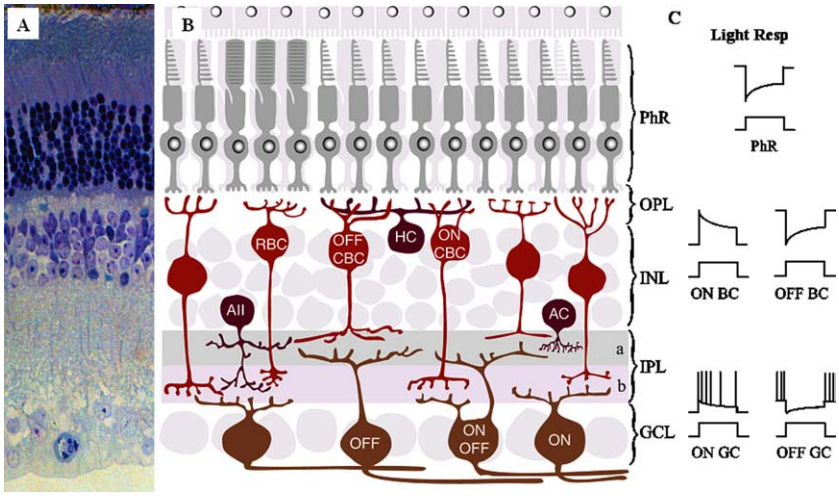

Fig. 1. *Cellular structure of retina.* (A) A cross section of an adult mouse retina. Note that the IPL takes 30% of the total thickness of the retina, in which RGCs synapse with bipolar and amacrine cells. (B) Schematic drawing of the principal anatomical components and synaptic connections of retina. Photoreceptors (rods and cones) synapse with bipolar and horizontal cells in the OPL. RGCs synapse with bipolar and amacrine cells in the IPL. All ON RGCs synapse with bipolar cells in sublamina *b*, all OFF RGCs make synapse with bipolar cells in the sublamina *a* of the IPL. Subpopulation of RGCs receives synaptic inputs from both ON and OFF bipolar cells. Rod-driven bipolar cells synapse with AII amacrine cells, which in turn make electrical synapse with cone-driven OFF bipolar cells and glycinergic synapse with cone-driven ON bipolar cells. (C) Representative light responses of retinal neurons. Light responses in outer retinal neurons, such as photoreceptors, bipolar and horizontal cells, are graded potentials. In the inner retina transient signals and spikes originate on RGCs and amacrine cells. PhR, photoreceptor; AC, amacrince cell; AII, AII amacrine cell; HC, horizontal cell; GC, ganglion cell; Off CBC, cone-driven OFF bipolar cell; On CBC, cone-driven ON bipolar cell; RBC, rod-driven bipolar cell.

in distinct layers of the IPL. Despite the enormous diversity of the structural and functional properties among different subtypes of RGCs, all ON RGCs ramify only in sublamina *b* of IPL and synapse with ON bipolar cells. In contrast, all OFF RGCs ramify only in sublamina *a* of IPL and synapse with OFF bipolar cells (Famiglietti & Kolb, 1976; Nelson, Famiglietti, & Kolb, 1978). A subset of ON–OFF RGCs, however, ramifies their dendrites in both sublaminae and signals both the onset and the termination of light (Amthor, Oyster, & Takahashi, 1984). The rod-driven bipolar cells do not directly synapse with RGCs. Instead, they synapse with AII amacrine cells and depolarize these cells when light is on. The later then depolarizes cone-driven ON bipolar cells through a gap junction connection and hyperpolarizes cone-driven OFF bipolar cells and OFF RGCs by releasing inhibitory neurotransmitter, glycine, on these cells (Bloomfield & Dacheux, 2001). Therefore, the separation of rod-driven ON and OFF pathways starts at the connections between AII amacrine cells and cone-driven bipolar cells. In adult retina, the photoreceptor-bipolar-ganglion cell vertical pathway provides principal excitatory synaptic inputs carried by ribbon synapses. The conventional synapses in the outer plexiform layer (OPL) and the IPL of retina formed by horizontal and amacrine cells provide inhibitory synaptic inputs.

3. Development of retinal synaptic pathways

In most mammals, retinal neurogenesis and synaptogenesis start before birth and continue during early postnatal development. By eye opening, virtually all retinal cell types are born and most synaptic contacts are established. In rodents, the synaptic connections between photoreceptors and RGCs provided by bipolar cells, which synapse postsynaptic to photoreceptors in the OPL and presynaptic to RGCs in the IPL and thus provide the synaptic link necessary to elicit light responses in RGCs, are established slightly before eye opening (Marquardt & Gruss, 2002). Synaptogenesis continues for several weeks after the establishment of the synaptic connections from photoreceptor to RGCs. In mouse, the density of both ribbons and conventional synapses in the IPL continuously increases after eye opening (postnatal day 12–13, P12-13) and reaches the peak level by the age of P21 (Fig. 2A).

Consistent with the time course of synaptogesis in retina, the synaptic strength, measured as the frequency of spontaneous synaptic inputs, of retinal neurons continuously matures after eye opening. In mouse retina, the frequency of RGC spontaneous activity is low before and shortly after eye opening. A surge of glutamate receptor-mediated spontaneous excitatory postsynaptic currents (sEPSCs) and GABA/glycine receptor-mediated spontaneous inhibitory postsynaptic currents (sIPSCs) emerges around P25 (Fig. 2B), increasing RGC synaptic inputs over 4-fold in 2 weeks after eye opening and subsiding by P60 (Tian & Copenhagen, 2001). Amplitudes of RGC light responses in cat and ferret retina were also found increasing after eye opening (Tootle, 1993; Wang, Liets, & Chalupa, 2001).

RGC dendritic stratification into ON and OFF pathways is also regulated during postnatal development. In early postnatal developmental stage, the dendrites of RGCs in the initially established neural network are ramified throughout the IPL of retina (Bodnarrenko, Jeyarasasingam, & Chalupa, 1995; Bodnarrenko, Yeung, Thomas, & McCarthy, 1999; Diao, Sun, Deng, & He, 2004; Maslim & Stone, 1988; Wang et al., 2001). With increasing of age, most RGCs achieve their mature stratified patterns and restrict their dendrites only in sublamina *a* or *b* of the IPL. Using a transgenic mouse, in which a large variety of morphologically distinctive subtypes of RGCs were labeled with constitutively expressed yellow fluorescence protein (YFP), we

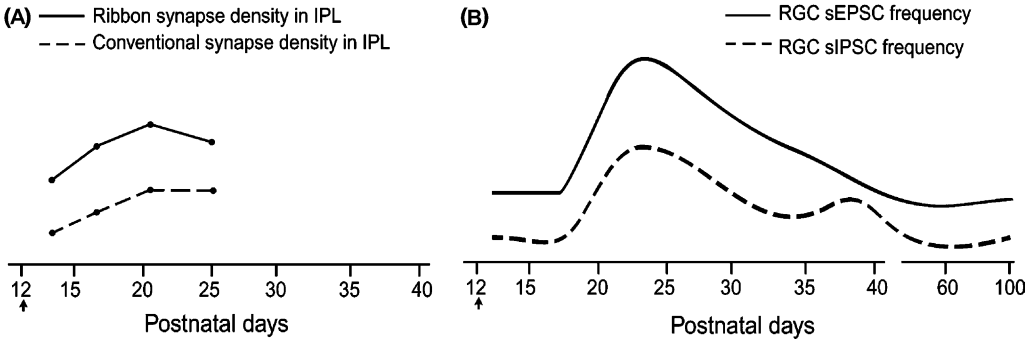

Fig. 2. *Synaptogenesis and maturation of synaptic activity in developing mouse retina.* (A) The density of both ribbon and conventional synapse in the IPL of mouse retina increase after eye opening and reach peak at the age of P21 (modified from Fisher (1979a)). (B) The frequency of RGC spontaneous synaptic inputs increases with age and reach peaks around 2 weeks after eye opening (modified from Tian and Copenhagen (2001)). The curves show the relative synaptic densities and frequencies of spontaneous synaptic inputs as functions of time in panels A and B, respectively. The numbers indicate the postnatal days. Arrows indicate the time of eye opening.

demonstrated that significant maturational changes in the ON and OFF pathways occur after eye opening at RGC level (Fig. 3, Tian & Copenhagen, 2003). Shortly before eye opening (P10), 53% of RGCs ramify in both sublamina *a* and *b* of the IPL. After eye opening, the proportion of multistratified RGCs significantly decreases. At the age of P30, only 29% of mouse RGCs ramify in both sublaminae (Fig. 4A). Functionally, the multistratified RGCs respond to both the onset and the offset of light stimulation. By recording light responses from a large number of neurons located in the mouse retina ganglion cell layer using a microelectrode array before and after eye opening (Fig. 4C), we found that the number of ON–OFF responsive cells declined more than 3-fold in two to three weeks after eye opening (Fig. 4B).

Consistent with the results from mouse retina, a recent study showed that the percentage of RGCs having light-evoked ON–OFF responses in ferret retina declined from ~40% prior to eye opening to ~20% after eye opening (Wang et al., 2001). Similar physiological and anatomical refinements have been observed in retina and higher centers of visual system of other mammals. A reduction in the proportion of bistratified RGCs has been observed in ferret and kitten (Bodnarrenko et al., 1995, 1999). In kitten, the percentage of ON–OFF responding cells in LGN decreases postnatally (Daniels, Pettigrew, & Norman, 1978). It is a strong inference that the ON–OFF responding RGCs must receive synaptic inputs from presynaptic neurons arborizing in both sublamina *a* and *b* of the IPL (Famiglietti & Kolb, 1976; Nelson et al., 1978). Therefore, the loss of light-evoked ON–OFF responsive RGCs would be commensurate with the reduction in the number of bistratified RGCs.

Mechanistically, the maturational alterations of ON and OFF pathways that occur after eye opening likely reflect refinements of the postsynaptic elements of bipolar/RGC synapses. It is postulated that RGCs achieve their mature stratified pattern by removing "misplaced" dendrites and restrict their dendrites only in sublamina *a* or *b* during development. This "pruning" of RGC dendrites is one of the best examples of the maturational reorganization of neuronal processes (Wong & Ghosh, 2002) and has been found in cat (Bodnarrenko et al., 1995; Dann et al., 1988; Maslim & Stone, 1988), ferret (Bodnarrenko et al., 1999), rabbit (Wong, 1990), rat

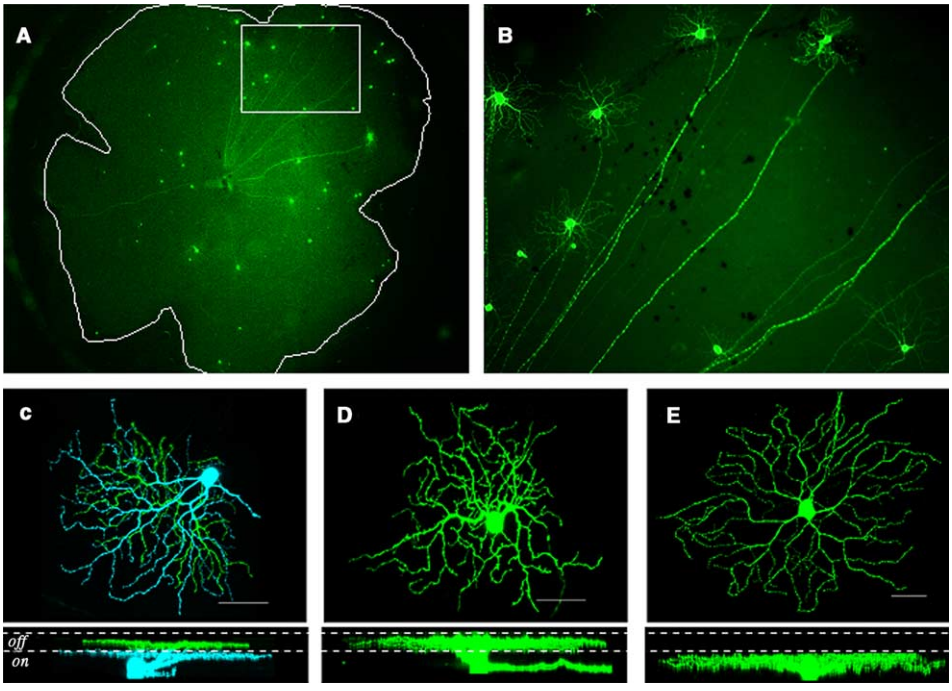

Fig. 3. *Dendritic ramification patterns of RGCs can be readily identified using confocal microscopy.* (A) View from vitreal side of a flat mounted retina harvested from a P30-aged, control reared Thy1-YFP expressing mouse. A rabbit polyclonal antibody for GFP conjugated with Alexa Fluor 488 (Molecular Probes, Inc., Eugene, OR) was used to enhance the YFP signal. A sheep polyclonal antibody for tyrosine hydroxylase (anti-TH, Chemicon International, Inc., Temecula, CA) was used to label dopaminergic amacrine cells. (B) An enlarged view of the area indicated by the box in panel A. Axons from individual RGCs cross the retina from each somata to the optic nerve head (modified from Tian and Copenhagen (2003)). (C, D and E) Upper panels show images of three representative mouse YFP-expressing RGCs from maximum projection of stacks of confocal images. Lower panels show 90° rotation views of the image stacks for the upper panels. YFP-expressing RGCs were classified into monostratified and bistratified cells based on their dendritic ramification within the IPL measured by examining the *z*-stack confocal imagings. The dendrites of bistratified ON–OFF RGCs (C) were clearly separated into two layers and ramified in both sublaminae *a* and *b* (color-coded as green and blue, respectively). Cells with their dendrites exclusively ramified in either sublamina *a* or *b* were classified as OFF or ON RGCs, respectively (D and E). Scale = 50 μm.

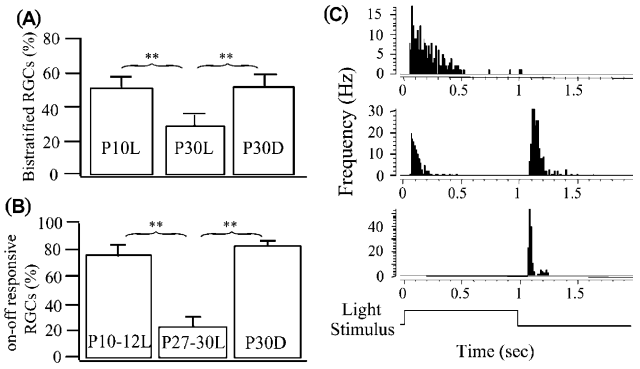

Fig. 4. *Light deprivation suppresses the refinement of RGC dendritic stratification in the IPL and functional maturation of ON/OFF pathways.* (A) Mean percentage of RGCs that bistratified in both sublaminae from three age groups of mice. P10L and P30L, mice raised in cyclic light/dark conditions from birth to P10 and P30, respectively. P30D, mice raised in constant darkness from birth to P30. The percentage of bistratified RGCs was 53 ± 2.7% (mean ± SE, *n* [number of retinas] = 4; total of 100 cells), 29 ± 3% (*n* = 5; 198 cells) and 53 ± 2.5% (*n* = 6; 396 cells) for the P10L, P30L and P30D groups, respectively. The differences between control reared mice at P10 versus P30 and control reared mice at the age of P30 versus dark reared mice at the age of P30 were statistically significant ($p < 0.0001$). The difference between control reared mice at the age of P10 and dark reared mice at the age of P30 was not significant ($p = 0.9277$). (B) Mean percentage of RGCs that responded to both the onset and offset of light stimulus in three age groups of mice. P10-12L and P27-30L, mice raised in cyclic light/dark conditions from birth to the ages of P10-12 and P27-30, respectively. P30D, mice raised in constant darkness from birth to the age of P30. The age-matched dark reared group had 4.4-fold more ON–OFF responsive RGCs than the P27-P30 control group. The difference was statistically significant ($p < 0.0001$). The difference between P10-P12 control group and the dark reared group was not significant ($p = 0.4006$). (C) Representative frequency histograms of RGC light responses of an ON cells (top), which had increased spike activity at the onset of light stimulus, an ON–OFF cell (middle), which had increased spike activity at both onset and offset of light stimulus and an OFF cell, which responded only to the offset of light (modified from Tian and Copenhagen (2003)). Error bars indicate standard errors in this and next figure.

(Yamasaki & Ramoa, 1993) and mouse (Tian & Copenhagen, 2003). Although the exact underlying synaptic and molecular mechanisms for the RGC dendritic stratification are not clear, recent studies suggest that this developmental remodeling process is crucially dependent on visually evoked synaptic activities after eye opening.

4. Effects of visual deprivation on the maturation of retinal synaptic pathways

Visual deprivation affects many functional and morphological attributes of retinal synaptic pathways. In mouse retina, light deprivation blocked the developmental surge of spontaneous synaptic inputs to RGCs and an age-dependent increase of inner retinal light responses after eye opening (Tian & Copenhagen, 2001; Vistamehr & Tian, 2004). The density of conventional synapse in the IPL of dark reared mouse and rat retina is greater than that of animals reared in cyclic dark/light conditions (Fisher, 1979b; Sosula & Glow, 1971). In dark reared turtle retina, receptive field areas of RGCs expanded to more than twice of those observed in normal reared animals (Sernagor & Grzywacz, 1996). In developing rat retina, the expression of brain-derived neurotrophic factor (BDNF), a factor that controls RGC dendritic arborization, is modulated by visual experience (Seki, Nawa, Fukuchi, Abe, & Takei, 2003). Dark rearing also blocked an age-dependent remodeling of dendritic complexity of a class of RGCs in hamster retina (Wingate & Thompson, 1994).

We recently examined the relative population of bistratified RGCs in dark reared mice and comparing the results with mice raised under cyclic light conditions. Our results showed that light deprivation completely blocked the age-dependent decline of bistratified RGCs (Tian & Copenhagen, 2003). The percentage of bistratified RGCs in mice raised in constant darkness was significantly higher than that of age-matched controls, but not different from that of the mice before eye opening (Fig. 4A). Consistent with the changes in RGC dendritic morphology, the maturational loss of functional ON–OFF responsive RGCs is absent in dark reared mice. The percentage of ON–OFF responsive RGCs dropped 4.4-fold from P10-12 to P30 and light deprivation retarded this developmental decline (Fig. 4B). Thus, light deprivation not only eliminated the developmental loss in the percentage of ON–OFF responsive cells physiologically but also retarded the "pruning" process that normally reduces the number of RGCs with bistratified dendrites anatomically.

Similar retardation in the loss of bistratified RGCs have been reported in earlier pharmacological studies in which intraocular injections of APB, the compound that specifically hyperpolarizes cone-driven ON bipolar and rod-driven bipolar cells and thereby prevents the release of glutamate by these cells halted the developmental stratification process of RGC dendrites (Bisti, Gargini, & Chalupa, 1998; Bodnarrenko & Chalupa, 1993; Bodnarrenko et al., 1995). Therefore, from a morphological point of view, light deprivation or intraocular injection of APB, which can theoretically mimic the effects of light deprivation on the ON pathway, can block the developmental refinement of RGC dendritic stratification.

5. Effects induced by light deprivation on retina are reversible in both young and adult animals but persistent in constant darkness

Although light deprivation induced pronounced effects on the maturation of RGC dendritic stratification,

frequency of RGC spontaneous synaptic inputs and retinal light responsiveness, some of the effects are found to be reversible by returning the animals back to the cyclic light/dark conditions (Tian & Copenhagen, 2001). Dark rearing of mice shortly after birth to P30 significantly reduced the inner retinal light responses measured as oscillatory potential (OPs) of electroretinogram (ERG) (Fig. 5). This reduction in OPs recovered to control levels in animals that were returned to cyclic light/dark conditions for 15 days (Fig. 5C). The suppressive action induced by dark rearing on the rate of RGC sEPSCs is also reversible (Fig. 5D). However, this recovery takes more than a week. This prolonged time period required for recovery is consistent with synaptic reorganization that requires gene transcription and/or changes in neuronal structure. The time course is much slower than the changes in glutamate receptor subunit expression that can be observed in visual cortex within hours of exposure to light following darkness (Quinlan, Philpot, Huganir, & Bear, 1999), but is comparable to the recovery of physiological cortical function in cats after monocular deprivation (Movshon, 1976).

To further determine whether there is a critical time period during which the retina shows evidence of increased plasticity, we have recently examined the effects of light deprivation on the light responsiveness of inner and outer retina at different ages using ERG measurements (Vistamehr & Tian, 2004). Our results showed that dark rearing mice from birth to P30, P60 and P90 persistently suppresses the inner retinal light responses measured as the amplitude of OPs. Light deprivation from birth also temporarily delays the developmental increase of the amplitudes of outer retinal light responses measured as the amplitudes of ERG a- and b-wave (Fig. 6). These suppressions of ERG amplitudes by light deprivation can be fully reversed by returning the animals to cyclic light/dark conditions. However, the time course of the recovery is age-dependent with younger animals requiring longer time for recovery. In addition, dark rearing of adult mice for 30 days produces the same degree of suppression on OPs, but not a- and b-wave, amplitude as age-matched mice dark reared from birth. These results demonstrated a degree of activity-dependent functional plasticity in the inner retina of both young and adult mouse, which has not been described previously. It is, however, still an open question how visual experience modulates the developmental changes of RGC synaptic strength, dendritic ramification and light responsiveness.

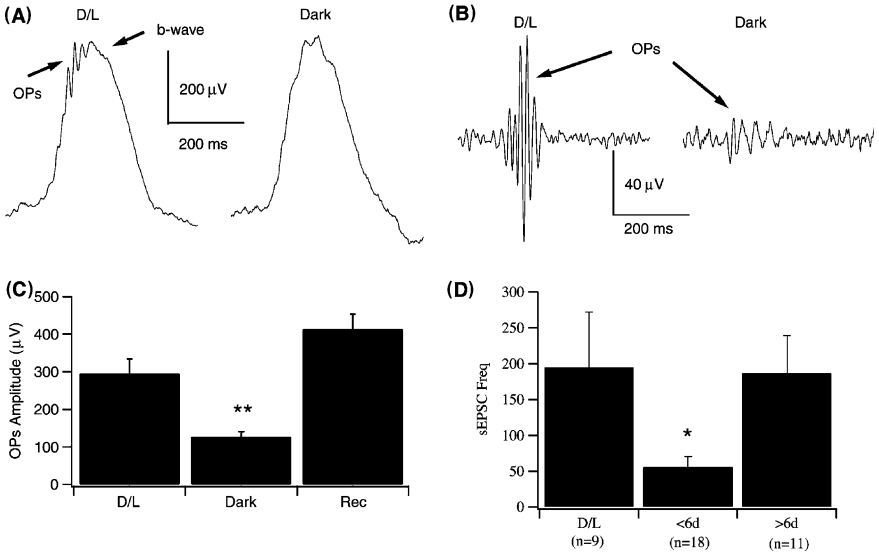

Fig. 5. *The effects induced by light deprivation on ERG OPs and RGC spontaneous synaptic inputs are reversible; recovery from dark rearing requires at least 6 days.* ERGs, recorded corneally, were compared among three groups. One group of animals was dark reared from P5 through P32 and recorded at P32 (Dark). Animals in the Recovery group (Rec) were dark reared from P5 and returned to cyclic light at P30. ERGs were recorded at P45. (A) Flash-evoked ERG waves recorded, respectively, from a cyclic light reared (D/L) and a dark reared (Dark) mouse. (B) OPs, isolated by bandpass filtering (73–500 Hz) the ERG waves of Fig. 5(A). (C) Average amplitudes of OP waves. Amplitudes were calculated by the sum of peaks (Severns et al., 1994). OP amplitudes were significantly lower in the Dark group compared to both D/L and Rec groups. Recovery was observed in the group of mice tested 15 days after return to cyclic light. The differences in OP amplitudes were highly significant statistically (D/L versus Dark: $p = 0.005$; Dark versus recovery: $p < 0.0001$). (D) sEPSCs were recorded from RGCs of animals raised either in a normal daily dark/light cycle (D/L group) or in darkness (P5 to P20) and subsequently recovered in cyclic light for 2–5 days (< 6d group) and 7–10 days (> 6d group). The frequency of sEPSCs remained significantly below cyclic light reared animals in the period up to 6 days after return to cyclic lighting. After more than 6 days of cyclic light, the sEPSC frequency of initially dark reared animals is very close to those of control animals. * indicates the difference is statistically significant and p value is between 0.05 and 0.01. ** indicates p value is smaller than 0.01. n indicates number of cells in each group (modified from Tian and Copenhagen (2001)).

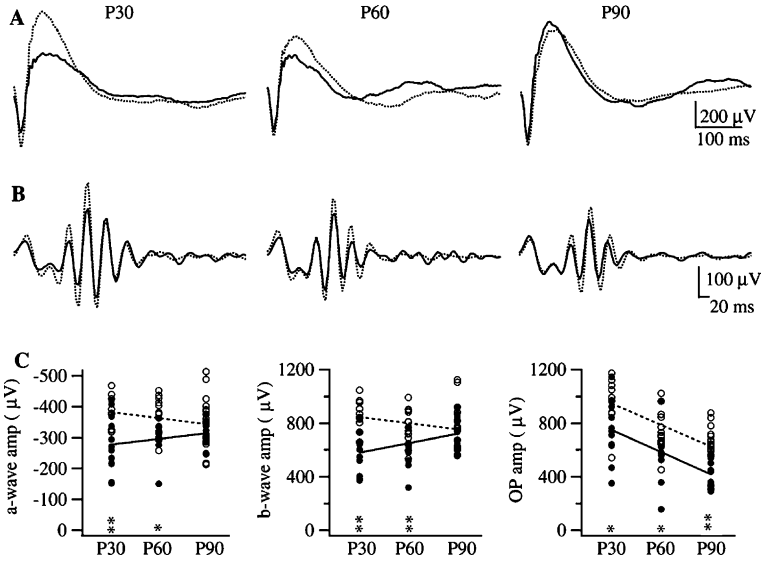

Fig. 6. *Light deprivation delays the maturational increase of a- and b-wave amplitudes but persistently suppresses the OP amplitudes.* (A) Representative ERG waveforms recorded from mice reared under constant darkness (solid line) and compared with ERGs from age-matched control animals raised under cyclic light/dark conditions (dotted line) at the ages of P30 (left), P60 (middle) and P90 (right). (B) Representative OPs waveforms recorded from mice raised under the same two conditions at the same three ages as in (A). (C) Scatter plots of the amplitudes of ERG a-wave (left), b-wave (middle) and OPs (right) measured from mice raised under cyclic light/dark conditions (open cycles) and constant darkness (filled cycles). The dotted and solid lines indicate the linear regression fittings of the amplitudes as a function of age from mice raised under the two different conditions, respectively. These results demonstrated that magnitudes of the suppression of a- and b-wave amplitudes induced by dark rearing are age dependent but the suppression of OPs is age independent (modified from Vistamehr and Tian (2004)).

6. The possible mechanisms of activity-dependent maturation of synaptic pathways in retina

Several possible mechanisms have been postulated for the refinement of ON/OFF pathways at RGC level. Some evidences showed that activity-dependent RGC elimination can regulate the maturation of ON and OFF pathways. This hypothesis is supported by the observation that the formation of mosaics distribution of ON and OFF RGCs during development is regulated by RGC activity. ON and OFF RGCs in mature retina are arrayed in regular mosaics, with adjacent cells commonly forming ON and OFF pairs (Wässle, Boycott, & Illing, 1981; Wässle, Peichl, & Boycott, 1981). By counting the number of all alpha RGCs in developing cat retina, it was shown that about 20% of these cells are eliminated during the first postnatal month. Computer simulation suggests that the loss of these "excess" cells, which mask the regular mosaic patterns in the early postnatal life, is sufficient for the formation of ON/OFF mosaic of adult retina. Block of voltage-gated sodium channel activity by intraocular injection of TTX altered the regular mosaic pattern of RGC distribution in a dose-dependent manner (Jeyarasasingam, Snider, Ratto, & Chalupa, 1998). However, TTX had little effect on the formation of stratified RGCs in cat retina (Campbell, Ramoa, Stryker, & Shatz, 1997; Wong, Herrmann, & Shatz, 1991).

It was also proposed that synaptic transmission from bipolar cells triggers an intrinsic program in multistratified RGCs leading to the retraction of one or another set of their dendritic processes. This model needs to find an ON- or OFF-RGC specific molecular composition, which are differentially expressed on the dendrites of immature multistratified RGCs (Bodnarrenko et al., 1995; Wang et al., 2001). Although there is no direct evidence to support this model, some experimental results suggest that ON and OFF dendritic stratification of RGCs are differently regulated. For instance, morphologically identified ON and OFF RGCs in ferret retina have different intrinsic excitability and develop different activity patterns during the period of ON–OFF segregation (Myhr, Lukasiewicz, & Wong, 2001; Wong & Wong, 2000), suggesting the mechanisms of internal control of cellular activity may be different between ON and OFF RGCs. However, how these mechanisms are influenced by light stimulation needs to be further elucidated.

One attractive hypothesis is that the visual experience-dependent maturation of ON/OFF pathways in retina might undergo a "push–pull" competition between ON and OFF dendrites on immature bistratified RGCs. This competition could be regulated by a NMDA receptor involved Habbian mechanism, which strengths synaptic connectivities with correlated activity and eliminates synapses firing asynchronously. Such a Hebbian plasticity will favorite immature bistratified

RGCs becoming either monostratified ON or OFF cells since ON or OFF inputs from any given ON or OFF bipolar cell will more likely synchronize with inputs from the same type of bipolar cells but not other types. Hebbian plasticity has been well documented in developing CNS. NMDA receptor-mediated current, which is sharpened over development by an activity-dependent switch in predominant subunit composition from NR2B to NR2A, is a key player in this mechanism. Consistent with this idea, NR1/NR2A and NR1/NR2B subunit-containing NMDA receptors are found on RGC dendrites (Fletcher, Hack, Brandstatter, & Wassle, 2000; Pourcho, Qin, & Goebel, 2001). These NMDA receptors can be activated by light-evoked release of glutamate from bipolar cells (Cohen, 2000; Diamond & Copenhagen, 1995; Mittman, Taylor, & Copenhagen, 1990). Similar to the developmental stratification of RGC dendrites, the expression of NR2A in developing RGCs is age-dependent. Dark rearing of 1 week at the age of P12 decrease the expression of NR2A in rat retina (Xue & Cooper, 2001). This activity-dependent switch over of NMDA subunit from NR2B to NR2A might contribute to our recent observation that the decay time constant of RGC light-evoked EPSCs decreased more than 50% from the ages of P10-14 to P30-34 and light deprivation blocks this age-dependent change in kinetics of RGC synaptic inputs (He & Tian, unpublished data). In addition, our recent study on *Spastic* mice, in which the expression of glycine receptors was reduced more than 80%, showed a significant reduction of RGCs ramified their dendrites in sublamina *a* of the IPL (Xu & Tian, 2004). An earlier study demonstrated that RGCs in these mutants have significantly reduced surround OFF responses (Stone & Pinto, 1992), suggesting that synaptic inputs from OFF bipolar cells are critical to the maturation of OFF pathway. Furthermore, light deprivation, genetic deletion of NR2A, pharmacological block of ON pathway activity in retina or artificially correlate ON and OFF RGCs activity disrupts the development of cortical cell orientation selectivity (Chapman & Gödecke, 2000; Fagiolini et al., 2003; Weliky & Katz, 1997), strongly suggesting that there is a link between visual experience, age-dependent expression of NR2A and maturation of ON/OFF pathways in retina and orientation selectivity in visual cortex.

For some RGCs, however, direct synaptic inputs from bipolar cells might not be necessary for the dendritic stratification. A significant percentage of RGCs are stratified even before bipolar cells form synapse with RGCs, suggesting mechanisms without bipolar cell input may direct the stratification of RGC during early development (Feller, 2002; Sernagor, Eglen, & Wong, 2001). It is thought that cholinergic amacrine cells may play a key role for the dendritic stratification of RGCs since they are among the earliest retinal neurons to become differentiated and their processes stratified in the IPL before those of RGC. Consistent with this notion, genetic deletion of β subunit of nicotinic acetylcholine receptors resulted in more wildly stratified dendritic arbors across the whole depth of the IPL in mouse RGCs (Bansal et al., 2000). In turtle retina, blockade of cholinergic transmission in vivo resulted in relatively smaller RGC dendritic arbors (Sernagor & Grzywacz, 1996).

Regardless how many different underlying mechanisms could regulate the refinement of retinal synaptic circuitry, it is increasingly clear that visual experience plays an important role in the maturation of retinal synaptic pathways, at least the ON and OFF pathways. More direct evidence from physiological, morphological and molecular levels will help to elucidate how the maturation of retinal synaptic pathways is regulated by what aspect of visual stimulation.

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