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Developmental mechanisms that regulate retinal ganglion cell dendritic morphology

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Abstract

One of the fundamental features of retinal ganglion cells (RGCs) is that dendrites of individual RGCs are confined to one or a few narrow strata within the inner plexiform layer (IPL), and each RGC synapses only with a small group of presynaptic bipolar and amacrine cells with axons/dendrites ramified in the same strata to process distinct visual features. The underlying mechanisms which control the development of this laminar-restricted distribution pattern of RGC dendrites have been extensively studied, and it is still an open question whether the dendritic pattern of RGCs is determined by molecular cues or by activity-dependent refinement. Accumulating evidence suggests that both molecular cues and activity-dependent refinement might regulate RGC dendrites in a cell subtype-specific manner. However, identification of morphological subtypes of RGCs before they have achieved their mature dendritic pattern is a major challenge in the study of RGC dendritic development. This problem is now being circumvented through the use of molecular markers in genetically engineered mouse lines to identify RGC subsets early during development. Another unanswered fundamental question in the study of activity-dependent refinement of RGC dendrites is how changes in synaptic activity lead to the changes in dendritic morphology. Recent studies have started to shed light on the molecular basis of activity-dependent dendritic refinement of RGCs by showing that some molecular cascades control the cytoskeleton reorganization of RGCs.

Keywords

retinal ganglion cells; retinal development; synaptic activity; dendritic morphology; synaptic connection

Introduction

Retinal ganglion cells (RGCs) are the output neurons of the retina. In the retina, RGCs synapse with bipolar and amacrine cells in the inner plexiform layer (IPL) to receive excitatory and inhibitory synaptic inputs. The axons of RGCs travel through the optic nerve to retinorecipient structures in the brain, where they transfer specific aspects of visual information to the higher centers of the visual system (Masland, 2001; Mu and Klein, 2004; Nassi and Callaway, 2009; Wässle, 2004). To achieve the functional specificity, it is crucial that dendrites of individual RGCs are confined to one or a few narrow strata within the IPL, and each RGC synapses only with a small group of presynaptic bipolar and amacrine cells with axons/dendrites ramified in the same strata to process distinct visual features. The synaptic circuitries processing distinct visual features are so called “parallel pathways”

(Coombs and Chalupa, 2008; Famiglietti and Kolb, 1976; Ghosh *et al.* 2004; Kuffler, 1953; Masland, 2001; Wässle, 2004). In most mammals, RGCs can be divided into about 20 morphological subtypes based on their distinctions in the dendritic structure and synaptic connections (Badea and Nathans, 2004; Berson, 2008; Coombs *et al.*, 2006; Dacey and Packer, 2003; Kong *et al.*, 2005; Rockhill *et al.*, 2002; Sun *et al.*, 2002; Völgyi *et al.*, 2009).

Several studies have shown that some RGCs initially extend their dendrites through multiple sublaminae and then refine their dendritic arbors to achieve a laminar-restricted pattern in cat, mouse and rat retina (Bodnarenko and Chalupa, 1993; Bodnarenko *et al.*, 1999; Chalupa and Günhan, 2004; Coombs *et al.*, 2007; Diao *et al.*, 2004; Maslim and Stone, 1988; Tian, 2008; Tian and Copenhagen, 2003; Xu and Tian, 2007; Yamasaki and Ramoa, 1993). Other studies showed that RGC dendrites can be confined into appropriate laminae directly without refinement in fish and rodents (Coombs *et al.*, 2007; Kim *et al.*, 2010; Mumm *et al.*, 2006; Yonehara *et al.*, 2008). Therefore, it is still an open question how the dendrites of RGCs become restricted to appropriate IPL sublaminae. Accumulating evidence suggests that molecular cues and activity-dependent refinement might both regulate RGC dendrites in a cell subtype-specific manner. However, it is unclear which subtypes of RGCs undergo developmental dendritic refinement, which subtypes of RGCs confine their dendrites directly and if there are any general rules that vary according to subtypes of RGCs. A major challenge in determining whether dendritic morphology and synaptic connections of different subtypes of RGCs develop in distinct manners is to identify morphological subtypes of RGCs before they have achieved their mature dendritic pattern. This limitation severely compromises analysis of RGC development. Recent development using molecular markers in genetically engineered mouse lines to identify RGC subsets enables us to classify RGC subtypes without mature dendritic morphology (Badea *et al.*, 2009; Hattar *et al.*, 2002; Huberman *et al.*, 2009; Kim *et al.*, 2008; 2010; Siebert *et al.*, 2009; Yonehara *et al.*, 2008). Another unanswered fundamental question in the study of activity-dependent refinement of RGC dendrites is how changes in synaptic activity lead to the changes in dendritic morphology. Recent studies have started to shed light on the molecular basis of activity-dependent dendritic refinement of RGCs by showing that some molecular cascades control the cytoskeleton reorganization of RGCs. In this short review, I will briefly summarize the recent advances in our understanding of how RGC dendritic morphology and synaptic connections are regulated during development and identify the possible molecular mechanisms.

Neurogenesis and synaptogenesis of retina

During retinal development, RGCs differentiate first followed by cones and horizontal cells. Amacrine cells and rods differentiate shortly afterward. Bipolar cells are the last neurons to differentiate. In mammals, most retinal neurons differentiate before birth (Altshuler *et al.* 1991; Cepko *et al.* 1996; Marquardt and Gruss, 2002). Neurogenesis of RGCs is largely determined intrinsically. Several genes have been identified to determine the neurogenesis of RGCs (Mu and Klein, 2008). For instance, genetic deletion of Pax6, a member of the homeobox gene family, math5, a member of the basic helix–loop–helix (bHLH) family, or *Barhl2*, a member of the *Barh* gene family, significantly reduces or completely blocks the differentiation of RGCs in mice (Brown *et al.* 2001; Dign *et al.*, 2009; Marquardt and Gruss, 2002; Marquardt *et al.* 2001; Wang *et al.* 2001a).

The order of synaptogenesis of retinal neurons is somewhat different from the order of neurogenesis. During development, synapses of amacrine cells in the IPL appear first. These are followed by the synaptic formation between photoreceptors and horizontal cells in the OPL. The last synaptic element to link photoreceptors in the outer retina and RGCs in the inner retina to elicit light responses in RGCs is the synaptic connection between bipolar cells

and RGCs (Stone *et al.* 1984; Nishimura and Rakic, 1987). It was postulated that molecular cues play a crucial role in retinal synaptogenesis for RGCs. Consistently, RGCs in mice and zebrafish retina have been found to ramify their dendrites into appropriate sublaminae directly without refinement (Coombs *et al.*, 2007; Kim *et al.*, 2010; Mumm *et al.*, 2006; Yonehara *et al.*, 2008). In addition, it was reported that members of the immunoglobulin superfamily adhesion molecules, DSCAMs and sidekicks, direct laminar-specific axonal and dendritic ramification of bipolar cells and RGCs in chick retina (Yamagata and Sanes, 2008) and RGC neurite arborization and mosaic formation in mouse retina (Fuerst *et al.* 2008). A recent study demonstrated that transmembrane semaphoring Sema6A and its receptor PlexinA4 (PlexA4) control the stratification of the dendrites of dopaminergic amacrine cells, melanopsin containing RGCs and calbindin-positive cells into ON and OFF sublaminae of the IPL in mouse retina (Matsuoka *et al.*, 2011).

However, many other studies have also shown that the dendritic morphology and synaptic connections of RGCs undergo profound refinement during postnatal development. Early in postnatal development, the dendrites of many RGCs ramify diffusely throughout the IPL of the retina in cats, rats and mice. With subsequent maturation, RGC dendrites become much more narrowly stratified in the IPL (Bansal *et al.* 2000; Bodnarenko and Chalupa, 1993; Bodnarenko *et al.* 1995, 1999; Coombs *et al.*, 2007; Diao *et al.* 2004; Kim *et al.*, 2010; Maslim and Stone, 1988; Wang *et al.* 2001b). In mouse retina, the width of RGC dendritic stratification decreases from 60% of the total IPL thickness at birth to approximately 20% of the total IPL thickness within 2 weeks after birth (Bansal *et al.* 2000; Coombs *et al.*, 2007; Diao *et al.* 2004; Xu and Tian, 2007). Part of this developmental decrease is attributed to the developmental restriction of RGC dendrites and part is attributed to the increase of the thickness of IPL (Coombs *et al.*, 2007). Therefore, this obvious discrepancy strongly suggests that different subtypes of RGCs might follow different processes for their dendritic maturation.

A recent study has shown evidence to reconcile this discrepancy. Kim *et al.* (2010) have investigated the development of RGC dendritic arbors using four transgenic mouse lines in which different subsets of RGCs are indelibly labeled with a fluorescent protein. Each subset has a distinct functional signature and the dendrites of each subset are restricted to specific sublaminae within the IPL in adulthood. These RGCs acquire their dendritic ramification patterns in different ways. For instance, direction selective ON-OFF RGCs directly ramify their dendrites into lamina-restricted bistratified pattern during the first postnatal week (Fig 1A). However, direction selective OFF RGCs and a subset of bistratified non-direction selective OFF RGCs initially ramify their dendrites diffusely throughout the whole thickness of IPL or the entire outer IPL during the first postnatal week. Then they restrict their dendrites into lamina-specific patterns by dendritic elimination during the second postnatal week (Fig 1B). Another subset of bistratified ON-OFF RGCs develops their dendrites stepwise. They form one layer of dendritic plexus in the center of IPL during the first postnatal week and add another layer of dendritic plexus in the outer IPL during the second postnatal week (Fig 1C). These results clearly demonstrate that RGCs could form their lamina-restricted dendritic pattern by targeted dendritic ramification, dendritic elimination and continuous dendritic growth.

Although all of the 4 different subsets of RGCs described above acquire their adult dendritic patterns before eye opening, other studies suggest that some RGCs remodel their dendrites after the retina receives light stimulation. Analysis of RGC dendritic arborization in mouse retinas before and after eye opening shows that 53% of RGCs ramify their dendrites in both sublamina *a* and *b* of the IPL and about 60% of these RGCs (roughly 30% of the total RGC population) have a single layer of narrowly stratified dendritic plexus ramified at the center of the IPL at the ages of P10-12. Functionally, these cells respond to both the onset and the

offset of light stimulation. Approximately 40% of these RGCs (roughly 20% of the total RGC population) ramify their dendrites in both sublamina *a* and *b* of IPL with two narrowly stratified dendritic plexuses. These so-called bistratified RGCs resemble the direction selective ON-OFF RGCs (Sun *et al.*, 2002; Völgyi *et al.*, 2009; Wei *et al.*, 2010). At the age of P33, only 11% of the total RGC population ramify their dendrites in both sublaminae *a* and *b* with one layer of narrowly stratified dendrites (Tian and Copenhagen 2003; Xu and Tian, 2007), suggesting a developmental redistribution of narrowly ramified RGC dendrites from one stratum to another stratum in the IPL (Fig 1D). This developmental redistribution of RGC dendrites in the IPL is also reflected physiologically as an age-dependent decrease of the number of RGCs that respond to both the onset and offset of light stimulation from 78% at P10-12 to 21% at the age of P27-30 (Tian and Copenhagen 2003). This maturational decline in the percentage of ON-OFF responding RGCs has also been observed in cat and ferret retinas (Bisti *et al.* 1998; Wang *et al.* 2001b). From a morphological point of view, this developmental redistribution of RGC dendrites in the IPL has to involve simultaneous adding of dendrites in one stratum and eliminating of dendrites in another stratum of the IPL. Consistent with this idea, a study of developing zebrafish retina using *in vivo* time-lapse imaging demonstrated that laminar-restricted dendritic plexus of RGCs could “migrate” from the inner border of the IPL to the outer border of the IPL in 2-3 days without being diffusely elaborated throughout the IPL (see figure 4C of Mumm *et al.*, 2006). However, the same technique has not been successfully applied to the study of developing mammalian retina and, therefore, it has not been directly demonstrated whether mammalian RGCs could take the same developmental strategy as that of zebrafish RGCs to redistribute their dendrites in the IPL.

In addition to the continuous refinement of RGC dendritic ramification in the IPL, other synaptic structures and functions of RGCs are reported to undergo continuous remodeling after the retina receives light stimulation. For example, the rate of spontaneous synaptic inputs to RGCs increases significantly in two weeks after eye opening in mice (Giovannelli *et al.*, 2008; Tian and Copenhagen 2001). The frequency of RGC light-evoked action potentials in cats, ferrets and mice increases after eye opening (He *et al.*, 2011; Tian and Copenhagen, 2001; Tootle 1993; Wang *et al.* 2001b) probably due to an age-dependent increase of the intrinsic excitability of RGCs (He *et al.*, 2011; Myhr *et al.*, 2001; Qu and Myhr, 2008), while the strength of light evoked synaptic inputs of RGCs measured as the light evoked synaptic currents decreases with age (He *et al.*, 2011).

AchR-mediated synaptic activity and dendritic maturation of RGCs

In an early developing vertebrate retina, RGCs fire periodic bursts of action potentials that are highly correlated and propagate across the RGC layer in a wave-like fashion (Wong 1999). These spontaneous retina waves are mainly mediated by excitatory neurotransmission with a developmental shift from cholinergic to glutamatergic synaptic transmission. In mice, retinal waves during the first postnatal week are mediated only by nicotinic acetylcholine receptors (nAChRs), while the retinal waves after P10-11 are mediated solely by glutamate receptors (GluRs). After the retina receives visual stimulation, the spontaneous retinal waves subside gradually (Bansal *et al.* 2000; Demas *et al.* 2003; Feller and Blankenship 2008; Feller *et al.* 1996; Xu *et al.*, 2010; Zhou 2001). It is well documented that both spontaneous synaptic activity before eye opening and light evoked retinal activity after eye opening are critical for the normal development of synaptic circuitry in higher centers of the visual system. Blocks of spontaneous or light evoked retinal activity disturb the development of eye-specific segregation of RGC axonal projection in the dLGN (Akeman *et al.* 2002; Chapman 2000; Grubb *et al.* 2004; Huberman *et al.* 2003; 2008; Muir-Robinson *et al.* 2002; Penn *et al.* 1998; Shatz and Stryker 1988; Torborg *et al.* 2005; Xu *et al.*, 2010), the fine-scale retinogeniculate projections (Hooks and Chen 2006), the visual map in superior

colliculus (Chandrasekaran *et al.* 2005; Huberman *et al.* 2008; McLaughlin *et al.* 2003; Mrsic-Flogel *et al.* 2005) and the precise thalamic projections to visual cortex (Cang *et al.* 2005; Rossi *et al.* 2001). However, the effect of synaptic activity on the development of RGC dendrites, especially RGC subtypes-specific effect, has not been fully characterized.

The effect of AChR-mediated retinal waves on the development of RGC dendritic morphology and synaptic connection has been documented. In the retina of turtle hatchlings, chronic blocks of nAChR-mediated retinal waves with curare reduced RGC receptive fields (Sernagor and Grzywacz 1996). In mice, genetic deletion of $\beta 2$ subunits of nAChR eliminated the retinal waves mediated by nAChRs during the first postnatal week and temporarily disturbed RGC dendritic stratification. The RGC dendrites of $\beta 2^{-/-}$ mice were not stratified or were only weakly stratified into two distinct sublaminae. Interestingly, the defects of RGC dendritic stratification are reversed after the GluR-mediated retinal waves start during the second postnatal week and the RGC dendrites segregated into four or five distinguishable strata as that of wild type animals (Bansal *et al.* 2000). However, the axonal projections of α -type OFF RGC to superior colliculus and dLGN and the thalamic projections to visual cortex of $\beta 2^{-/-}$ mice are permanently impaired (Cang *et al.* 2005; Huberman *et al.* 2008). On the other hand, it was also reported that genetic deletion of choline acetyltransferase (ChAT), the sole synthetic enzyme for acetylcholine (ACh), eliminates the retinal waves mediated by AChRs for the first few days after birth in mice but causes no detectable changes of dendritic morphology of RGCs and starburst amacrine cells (Stacy *et al.*, 2005).

GluR-mediated synaptic activity and dendritic maturation of RGCs

The effect of GluR-mediated retinal waves on the development of RGC dendritic restriction and synaptic connection is somewhat controversial. The earliest evidence demonstrating the critical roles of glutamatergic synaptic inputs from bipolar cells to the development of dendritic stratification and synaptic connection of RGCs was derived from a series of experiments by Bodnarenko and Chalupa (1993). During early postnatal development, intraocular injection of APB, which is an agonist for class III metabotropic GluRs (mGluR6) and hyperpolarizes rod bipolar cells and cone ON bipolar cells, resulting in a blockade of glutamate release from these neurons, caused an arrest of the developmental stratification and segregation of RGC dendrites into ON and OFF synaptic pathways. About 40% of the RGCs in APB treated adult retina have their dendrites multistratified in both sublaminae *a* and *b* of the IPL, which is significantly higher than that of untreated age-matched controls (Bodnarenko and Chalupa, 1993; Bodnarenko *et al.*, 1995; 1999) and this effect is irreversible with prolonged APB treatment (Deplano *et al.*, 2004). These results demonstrate that excitatory synaptic inputs from bipolar cells have a significant impact on the development of RGC dendritic stratification.

On the other hand, another recent study using transgenic mice in which the glutamate release from ON, but not OFF bipolar cells, is blocked by expressing tetanus toxin (TeNT) in ON bipolar cells driven by mGluR6 promoter. TeNT is a bacterial protease, which cleaves vesicle-associated membrane protein 2 (VAMP2) and inhibits vesicle fusion and transmitter release (Schiavo *et al.*, 1992). In TeNT expressing retina, the glutamate release from ON bipolar cells is selectively blocked and, therefore, the OFF RGCs have normal spontaneous and light evoked synaptic activity while the ON RGCs have very weak synaptic inputs. Surprisingly, the dendrites of neither ON nor OFF RGCs of TeNT mice are different from those of control mice although the number of the synapses between the ON bipolar cells and ON RGCs is smaller due to a reduced rate of synapse formation. Therefore, it was concluded that synaptic activity regulates synaptic formation, but not elimination, and synaptic density, but not dendritic morphology, in an ON-OFF pathway independent manner (Kerschensteiner

et al., 2009). In addition, genetic deletion of the mGluR6 receptor, which blocks ON bipolar cell light evoked synaptic activity, failed to impair dendritic stratification of mouse RGCs (Tagawa *et al.*, 1999). These results challenge the idea that glutamate released from ON bipolar cells is required for the normal development of RGC dendrites.

More recently, Xu *et al.* (2010) have shown that pharmacological blockade of GluRs by intraocular injection of antagonists for NMDA and AMPA receptors, AP5 and NBQX, significantly impairs the development of RGC dendritic morphology and synaptic function. Using time-lapse imaging, it was shown that the dendrites of mouse RGCs undergo very active remodeling at the age between P10-13. More than 30% of the dendritic protrusion is replaced every hour by continuous dendritic growth and elimination. Pharmacological blockade of GluR-mediated activity reduces the kinetic of RGC dendritic growth and elimination by approximately 50% and increases the dendritic density by 6-fold after 5 days treatment. The disrupted GluR-mediated activity in retina during early postnatal development is associated with profound and permanent defects of RGC dendritic morphology and synaptic function in adults, demonstrating the biological significance of GluR-mediated activity in RGC dendritic maturation. These results seem to be GluR-specific because intraocular injection of saline causes no detectable defect. Similarly, Lau *et al.* (1992) demonstrated that a blockade of NMDA receptors before eye opening increases the spine density of RGCs in hamsters.

It is not clear why pharmacological blockade of GluR-mediated activity causes significant dendritic defects while genetic inhibition of glutamate release from ON bipolar cells has a minimal effect on RGC dendrites. One possibility is that glutamate secreted from migrating neurons or from transient photoreceptor synapses in the IPL may modulate RGC development before bipolar cell synapses with RGCs (Wong *et al.*, 2000). This is supported by the evidence that the amplitude of AChR-mediated retinal waves is reduced in the presence of ionotropic GluR antagonists even before the transition from AChR-mediated retinal waves to GluR-mediated retinal waves occurs in normal mice (Bansal *et al.*, 2000). Another possibility is that different subtypes of RGCs undergo different maturational processes during postnatal development. Some RGCs, such as direction selective ON-OFF RGCs, form their laminar-restricted dendritic plexus without refinement early during postnatal development while others, such as direction selective and non-direction selective OFF RGCs, undergo profound dendritic refinement during the second postnatal week in mouse retina (Kim *et al.*, 2010). This later refinement might be sensitive to GluR-mediated synaptic activity. In addition, different subtypes of RGCs might have different sensitivity to glutamate during postnatal development. It was reported that GluR-mediated spontaneous synaptic inputs can be recorded from mouse RGCs as early as P7 (Johnson *et al.*, 2003) although the GluR-mediated retinal waves occur after P10 in mouse retina. It was also noted that GluR antagonists do not block retinal waves of the overall cell population in P8–P11 mouse retina, but they do block the rhythmic depolarization recorded by whole-cell current clamp in individual α -type RGCs (Bansal *et al.*, 2000). Furthermore, it is not clear whether glutamate released from OFF bipolar cells is sufficient to drive GluR-mediated retinal waves and regulate RGC maturation in TeNT and mGluR6^{-/-} mice.

Light evoked synaptic activity and dendritic maturation of RGCs

The effect of light evoked synaptic activity on the development of RGC dendritic restriction and synaptic connection seems also to vary among subtypes of RGCs and selective to some synaptic features. Functionally, light deprivation affects the expression of BDNF, a factor that controls RGC dendrite arborization, in rats (Seki *et al.*, 2003). In mice, light deprivation blocks the surge of spontaneous synaptic inputs to RGCs and an age-dependent increase of inner retinal light responses measured by ERG oscillatory potentials (Tian and Copenhagen

2001; Vistamehr and Tian, 2004), and impairs the maturation of the size of inhibitory receptive field of RGCs (Di Marco *et al.*, 2009). Morphologically, dark rearing blocks an age-dependent remodeling of dendritic complexity of a class of “aberrant” RGCs in hamster retina (Wingate and Thompson 1994). In mice, light deprivation increases the density of conventional synapses in the IPL (Fisher 1979a). The developmental ramification of RGC dendrites into OFF lamina of the IPL is selectively impaired by light deprivation in RGCs of mouse retina. However, light deprivation has no detectable effect on ON RGCs (Xu and Tian, 2007), suggesting the OFF RGCs are more sensitive to synaptic activity evoked by light stimulation. Similarly, long-term treatment of cat eyes with intraocular injection of APB significantly reduced the number of α RGCs ramifying in the sublamina *a* and increased the number of multistratified α cells (Deplano *et al.*, 2004). These results are in agreement with the recent study conducted by Kerschensteiner *et al.*, (2009) showing that blockade of ON RGC synaptic inputs has no effect on the dendritic morphology of RGCs in TeNT transgenic mice.

To further determine the role of light evoked synaptic activity from OFF pathway on OFF RGC dendritic maturation, Xu and Tian (2008) examined the relative population of morphologically and functionally identified RGCs in *Spastic* mice, in which the expression of glycine receptors is reduced by more than 80% (Becker *et al.* 1986). In mammalian retina the OFF signal from rod bipolar cells is conducted to OFF RGCs through glycinergic synapses between OFF bipolar cells/OFF RGCs and AII amacrine cells (Dacheux and Raviola 1986; Smith *et al.* 1986; Strettoi *et al.* 1990; Vaney *et al.* 1991; Wässle *et al.* 1995). Reduced expression of glycine receptors preferentially decreases the RGC OFF synaptic inputs with little effect on the ON response (Stone and Pinto 1992, Xu and Tian 2008). Before eye opening (P12), the ramification pattern of RGC dendrites of *Spastic* mice is indistinguishable from that of WT controls, suggesting that mutation of glycine receptors has no effect on the RGC dendritic maturation before retina is stimulated by light. However, the developmental redistribution of RGC dendrites from the center to the sublamina *a* of the IPL after eye opening was absent in *Spastic* mutants, which mimicked the effects induced by light deprivation on wild type animals. In addition, light deprivation of the *Spastic* mutants had no additional impacts on the RGC dendrites (Xu and Tian 2008), supporting the idea that a light evoked OFF signal is critical for the developmental redistribution of RGC dendrites from the center to sublamina *a* of the IPL. However, it is uncertain which subtypes of OFF RGCs are more sensitive to light deprivation because the depth of dendritic ramification in the IPL is commonly used to identify the morphological subtypes of RGCs (Sun *et al.*, 2002; Völgyi *et al.*, 2009). The development of new subtype-specific molecular markers is necessary to provide more unequivocal evidence for the classification of RGCs in immature retina.

It is increasingly clear that RGCs are heterogeneous not only in structure and function but also in the underlying regulatory mechanism for their dendritic and synaptic maturation. Some RGCs form their dendrites and synaptic connections directly without refinement and activity-dependent remodeling. The most extensively studied RGC subtype is the direction selective ONOFF RGC. These cells form their laminar-restricted dendritic pattern early during development even before they receive GluR-mediated synaptic inputs from bipolar cells (Kim *et al.*, 2010). Therefore, the dendritic structure and synaptic function of these cells are barely affected by blockade of bipolar cell glutamate release or light deprivation (Chan and Chiao, 2008; Elstrott *et al.*, 2008; Kerschensteiner *et al.*, 2009). It was postulated that the dendritic and synaptic formation of these cells could be regulated by AChR-mediated synaptic activity of cholinergic amacrine cells because the dendrites of these RGCs are closely associated with those of cholinergic amacrine cells and their functionality critically depends upon the synaptic inputs from cholinergic amacrine cells (Demb, 2007; He *et al.*, 1997; Yoshida 2001). However, ablation of cholinergic amacrine cells did not

influence the stratification of amacrine and bipolar cells (Reese *et al.*, 2001; Gunhan *et al.*, 2002). Although genetic deletion of the β subunit of nicotinic AChRs resulted in more wildly stratified dendritic arbors across the whole depth of the IPL in mouse RGCs (Bansal *et al.*, 2000), neither genetic deletion of the β subunit of nAChRs nor pharmacological blockage of synaptic activity mediated by AChRs affects the functionality of direction selective ON-OFF RGCs (Elstrott *et al.*, 2008; Wei *et al.*, 2011), demonstrating that the development of the synaptic circuitry of these cells does not require AChR-mediated spontaneous synaptic activity.

The dendritic structure and synaptic function of other RGCs might undergo significant developmental refinement. The developmental refinement of some of these RGCs is regulated by synaptic activity but others might not necessarily be sensitive to synaptic activity. Many studies have shown that the structural and functional properties of synaptic circuitry, such as the size of receptive fields of RGCs, the light-evoked synaptic output of RGCs, the 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 in cat, ferret and mouse retina (Bowe-Anders *et al.*, 1975; Deplano *et al.*, 2004; Giovannelli *et al.*, 2008; He *et al.*, 2011; Myhr *et al.*, 2001; Qu and Myhr, 2008; Rusoff and Dubin, 1977; Tian and Copenhagen, 2001; 2003; Tootle, 1993; Wang *et al.*, 2001b). Visual deprivation affects only some of the structural and functional features of RGCs, such as the number of synapses in the inner plexiform layer, the inhibitory receptive field, and the strength of RGC spontaneous synaptic inputs (Di Marco *et al.*, 2009; Fisher, 1979a; Giovannelli *et al.*, 2008; Sosula and Glow, 1971; Tian and Copenhagen, 2001), but not others, such as the cell number, size or staining characteristics of monkey RGCs, the dendritic field diameters, branching patterns, or total lengths of type I RGC dendrites of hamsters, the morphology of dendrites or axons of α or β RGCs of cats, the amplitude of ERG b-wave of cats, the conduction velocities, relative numbers and receptive field properties of X and Y cells and soma sizes of RGCs of cats, the direction selectivity, the receptive field mosaics, the light-evoked synaptic output and the intrinsic excitability of mouse and ferret RGCs (Anishchenko *et al.*, 2010; Baro *et al.* 1990; Chan and Chiao, 2008; Elstrott *et al.*, 2008; He *et al.*, 2011; Hendrickson and Boothe, 1976; Lau *et al.* 1990; Leventhal and Hirsch 1983; Sherman and Stone, 1973).

The possible mechanisms of developmental regulation of RGC dendrites

During developmental refinement, the dendritic arborizations of RGCs undergo dynamic elaboration, maintenance or elimination to attain the lamina-restricted ramification pattern. Although neuronal activity influences this remodeling in many subtypes of RGCs, it is unclear how activity exerts its effects. Several studies suggest that calcium seems to be important to link the neuronal activity with dendritic growth and patterning (Wong and Ghosh, 2002). It was reported that synaptic-stimulation-induced calcium influx through voltage-dependent calcium channels is sufficient to activate a transcriptional program that regulates the dendritic growth (Redmond *et al.*, 2002). The trans-membrane calcium influx activates a fast calcium/calmodulin dependent protein kinase IV (CaMKIV) pathway, which in turn phosphorylates cAMP-responsive element binding protein (CREB). Activation of this fast CaMKIV-CREB dependent signaling pathway could regulate gene expression of neurons. In addition, the trans-membrane calcium influx could activate a slower Ras/mitogen-activated protein kinase (MAPK) dependent pathway (Wu *et al.*, 2000, 2001), which ultimately regulates dendritic morphology. Consistent with this idea, a rise in calcium in the dendritic arborization can be confined to structures as small as dendritic spines (Yuste *et al.*, 2000). Using a calcium imaging approach, Lohmann *et al.* (2002) simultaneously monitored dendritic activity and structure in the intact retina. They found that blockade of calcium activity restricted to small dendritic segments, but not the overall increase of

calcium activity throughout the cell, caused rapid retraction of RGC dendrites. Therefore, they concluded that local calcium release is a mechanism by which afferent activity can selectively and differentially regulate dendritic structure across the developing arborization. Furthermore, activation of CaM kinase IV and CREB could also potentially activate brain-derived neurotrophic factor (BDNF), which had been reported to regulate dendritic growth in many parts of CNS.

BDNF/TrkB has been shown to play an essential role in the activity-dependent development of RGC dendrites (Landi *et al.*, 2007). Activation of BDNF promotes the anatomical segregation of the dendrites of ON- and OFF-center RGCs in different sublaminae of the IPL (Liu *et al.*, 2007; Landi *et al.*, 2007), while deletion of TrkB strongly inhibits visual experience-dependent refinement of RGC dendrites (Liu *et al.*, 2007). In addition, the expression of BDNF in the retina is up-regulated in a visual stimulation-dependent manner (Pollock *et al.*, 2001; Seki *et al.*, 2003; Mandolesi *et al.*, 2005; Landi *et al.*, 2007), suggesting that light deprivation retards the RGC dendritic maturation through reduction of the expression of BDNF. Consistently, over-expression of BDNF precludes the retardation of laminar refinement in dark-reared mice (Liu *et al.*, 2007). However, a fundamental unanswered question is how changes in synaptic activity lead to the changes in RGC dendritic morphology, which inevitably requires stabilization or reorganization of RGC cytoskeleton.

Recent studies demonstrated that genes typically associated with the immune system, such as those in the major histocompatibility complex (MHC), are expressed by neurons in various regions of the CNS, including retina, and may play important roles in synapse formation (Baudouin *et al.*, 2008; Corriveau *et al.*, 1998; Huh *et al.*, 2000; Ishii *et al.*, 2003; Syken and Shatz, 2003; Syken *et al.*, 2006; Xu *et al.*, 2010). Genetic deletion or mutation of a number of MHC class I genes, including $\beta 2$ -microglobulin, a MHCI cosubunit; or CD3 ζ , a key component of MHCI receptors; result in the failure of eye-specific segregation of RGC axon projections to the dLGN (Huh *et al.*, 2000; Xu *et al.*, 2010). In addition, genetic deletion of MHCI molecules enhances long-term potentiation (LTP) and abolishes long-term depression (LTD) in hippocampus (Huh *et al.*, 2000) and increases the frequency of spontaneous miniature synaptic currents in hippocampal and cortical neurons (Goddard *et al.*, 2007). Furthermore, spatial learning, memory, and neurogenesis in hippocampus are markedly reduced in immune-deficient mice (Ziv *et al.*, 2006), strongly suggesting that a common immune-associated mechanism might regulate various aspects of activity-dependent synaptic development and plasticity in the CNS.

Consistently, Xu *et al.*, (2010) reported that CD3 ζ is specifically expressed by RGCs and co-localized with other synaptic structural proteins, such as PSD95, in the IPL. Similar to the pharmacological blockade of GluR-mediated activity, genetic mutation of CD3 ζ profoundly reduces the kinetics of RGC dendritic growth and elimination, increases the number of RGC protrusion and dendritic branches and impairs the lamina-specific segregation of RGC dendrites in the IPL. In addition, CD3 ζ ^{-/-} mice show a selective reduction of GluR-mediated synaptic transmission of RGCs before eye opening and GluR antagonists have no additional effect on the RGC dendrites of CD3 ζ ^{-/-} mice. These findings strongly suggest that CD3 ζ -mediated signaling participates in activity-dependent synaptic maturation of RGCs in developing retina.

How could MHCI or CD3 ζ mediated signaling regulate RGC dendritic development? It was postulated that activation of immune molecules in neurons could produce similar intracellular signals as those generated in immune cells but with different ultimate effects, such as altering synaptic development, strength, neuronal morphology or circuit properties downstream of synaptic activity (Boulanger *et al.* 2001; Furgeaud and Boulanger 2007;

Stevens *et al.* 2007; Syken *et al.* 2006; Xu *et al.*, 2010). In the immune system, activation of CD3 ζ triggers several downstream cascades, including a Ras-MAPK pathway and actin-based cytoskeleton reorganization, which regulates immune cell polarization, migration and dendritic growth (Baniyash *et al.*, 2004; Kiefer *et al.*, 2002; Smith-Garvin *et al.*, 2009). It has been shown that most components of these cascades are expressed in CNS and implicated in activity-dependent synaptic activity (Shatz, 2009; Boulanger *et al.*, 2001). In addition, direct activation of CD3 ζ on hippocampal neurons affects cell morphology by promoting dendritic pruning through a tyrosine-based phosphorylation signaling motif common to the immune system (Baudouin *et al.*, 2008). Furthermore, MHC I proteins in neurons may interact with non-immune-proteins through non-classical signaling pathways (Ishii *et al.*, 2003; Ishii and Mombaerts, 2008). However, many more important questions, such as how MHC/CD3 ζ -mediated signaling interacts with neurotransmitter-mediated synaptic activity in dendritic maturation of neurons, what are the exact molecule mechanisms with which activation of MHC/CD3 ζ on neurons affects the maturation of dendrites, and whether MHC/CD3 ζ -mediated signaling specifically affects GluR-mediated synaptic transmission of neurons, need to be further addressed. Successfully addressing these questions will help us ultimately understand how changes in synaptic activity are translated into changes in neuron structure in developing retina.

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Fig 1. Schematic diagram illustrating four patterns of dendritic development

A. Targeted ramification. RGCs directly stratify their dendrites into laminar-restricted pattern during initial dendritic ramification with little further refinement. **B.** Dendritic elimination. RGCs initially ramify their dendrites diffusely in the IPL and then restrict their dendrites into laminar-restricted pattern by dendritic elimination. **C.** Continuous dendritic growth. RGCs form their laminar-restricted dendritic plexus stepwise by continuous dendritic growth after the initial dendritic ramification. **D.** Dendritic redistribution. RGCs form their laminar-restricted dendritic plexus in one stratum of the IPL during the initial dendritic ramification and then redistributed the dendritic plexus into another stratum during dendritic refinement by simultaneous and selective dendritic growth and elimination.