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Development of Retinal Ganglion Cell Structure and Function

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Abstract—In this review, we summarize the main stages of structural and functional development of retinal ganglion cells (RGCs). We first consider the various mechanisms that are involved in restructuring of dendritic trees. To date, many mechanisms have been implicated including target-dependent factors, interactions from neighboring RGCs, and afferent signaling. We also review recent evidence showing how rapidly such dendritic remodeling might occur, along with the intracellular signaling pathways underlying these rearrangements. Concurrent with such structural changes, the functional responses of RGCs also alter during maturation, from sub-threshold firing to reliable spiking patterns. Here we consider the development of intrinsic membrane properties and how they might contribute to the spontaneous firing patterns observed before the onset of vision. We then review the mechanisms by which this spontaneous activity becomes correlated across neighboring RGCs to form waves of activity. Finally, the relative importance of spontaneous versus light-evoked activity is discussed in relation to the emergence of mature receptive field properties.

1. INTRODUCTION

Retinal ganglion cells (RGCs) process and convey information from the retina to visual centers in the brain. These output neurons comprise subpopulations with distinct structure and function. The morphology of RGCs is highly disparate; their somata and dendritic field vary in size, and they exhibit strikingly varied dendritic architecture (Cajal, 1893; Wässle and Boycott, 1991; Rodieck, 1998) and axonal projection patterns (Friedlander and Tootle, 1990; Garraghty and Sur, 1993; Yamagata and Sanes, 1995a,b). Functionally, RGCs differ in their response to light in a variety of ways (reviewed by Wässle and Boycott, 1991; Rodieck, 1998; Dacey, 1999). Their response to light may be transient or sustained, brisk or sluggish, tonic or phasic. Some RGCs are good motion detectors and may prefer a specific direction of stimulus movement, whereas others are sensitive to the orientation of the stimulus but not its direction. In addition, RGCs show different contrast sensitivity, visual acuity, and color-coding. Despite the enormous diversity in structure and function, combined anatomical and electrophysiological studies have revealed a close correlation between the morphology and function of RGCs in vertebrates (Saito, 1983; Stanford and Sherman, 1984; Amthor *et al.*, 1984, 1989a, b; Dacey, 1999).

Within a species, structure–function studies have enabled classification of RGCs into broad subclasses (see Cook, 1998). For example, in the well-studied cat retina, small-field beta RGCs are the anatomical correlate of physiologically identified brisk-sustained or X-RGCs, and large-field alpha RGCs are correlated with brisk-sustained or Y-RGCs (reviewed by Wässle and Boycott, 1991). Major subclasses of RGCs, such as the alpha and beta cells in cat, can be further divided into subtypes, notably those which are depolarized (ON RGCs), or hyperpolarized (OFF RGCs), by light. In general, within a species, each subtype of RGC shares key features: (i) their dendritic branching patterns and arbor size are similar at any fixed retinal location; (ii) their dendritic fields overlap forming mosaics that cover the retinal surface effectively (Wässle *et al.*, 1983; Cook and Chalupa, 2000); (iii) they receive the same complement of presynaptic inputs; (iv) they project to common regions within targets in the brain. But not all RGC subclasses defined within one species are present in all species. However, in all species studied thus far, the inner plexiform layer (IPL), the plexus within which RGCs form intraretinal connections, is organized into structurally and functionally distinct sublaminae (Fig. 1). Irrespective of RGC subclass, ON RGCs have dendritic arbors that stratify in the inner region (sublamina b)

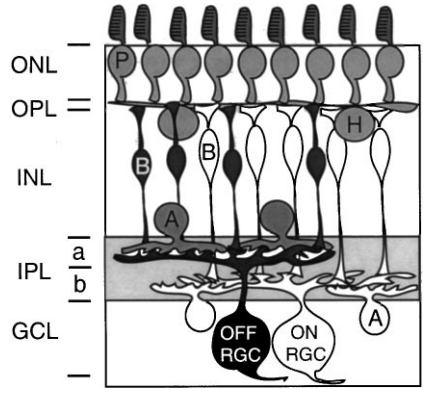

Fig. 1. Schematic representation of the retina. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; RGC, retinal ganglion cell; A, amacrine cell; B, bipolar cell; H, horizontal cell; P, photoreceptor. RGCs can be functionally divided into ON and OFF subclasses. The dendritic arbors of ON and OFF RGCs stratify in different sublaminae (a, OFF or b, ON) of the IPL.

of the IPL, whereas OFF RGCs stratify in the outer sublamina (sublamina a) of the IPL (Famiglietti and Kolb, 1976; Nelson *et al*., 1978). Cells with arbors in both sublaminae have ON and OFF responses (e.g. Amthor *et al*., 1984).

The diverse morphological and physiological properties of RGCs have presented an enormous challenge to investigators seeking to understand how the visual image is encoded and relayed to the brain. For developmental neurobiologists, the rich diversity of RGC structure and function make these neurons ideal for studies of cell-fate determination (reviewed by Cepko *et al*., 1996; Harris, 1997; Rapaport and Dorsky, 1998) and axonal and dendritic development (Goodman and Shatz, 1993; Wong, 1999a; Wong and Wong, 2000). Here, we have chosen to focus primarily on the structural and functional development of RGCs in a variety of species (see Table 1 for a summary of the developmental periods of some of these events). In recent years, there has been an increasing interest in obtaining a more unified view of the coordinated development of structure and function in RGCs. Although a complete understanding is still far off, many studies in the last decade have provided important insights into

Table 1. Timetable of the major events of RGC development across the species studied in this review*

Event	Ferret	Cat	Rat	Chick	Mouse	Turtle	Rabbit
Birth/hatching (days)	42 [1]	63 [1]	21–22 [1]	21 [2]	20 [3]	60 [4]	31–32 [3]
Duration of RGC genesis	E22–32 [5]	E21–36 [6]	E14–20 [7]	E2–8 [8]	E11–19 [9]		E13–31 [3]
Peak period of cell death	<P0–6 [10]	E36–60 [11,12]	P3–5 [13,14]	E12–15 [15]	P2–5 [16]		E31–32 [3]
Eye opening	P30–33 [1]	P7–9 [1]	P16–18 [17]	N/A	P10–12	N/A	P10–11
Onset of dendritic outgrowth/IPL first observed	<P0 [18]	E36 [19]	E17 [19]	E7 [2]	E17 [20,21]	E35	E24 [22]
Axons first innervate/reach targets	<P0 [23]	E32 [24]	E16 [25]	E6 [2]	E16 [26]	E35	E21 [27]
Duration of correlated bursting activity	<P0–25 [28]	<E52–P1> [29]	<E17–21> [30]	E8–18 [31,32,33]	<E17–P5> [34]	E40–P30 [4]	<P0–6> [35]
Emergence of light responses	P23 [36]	P3–4 [37]		E19 [2]		E40–45 [4]	P8 [38]

*All dates, except for birth/hatching, are given in terms of embryonic (E) and postnatal/posthatching (P) days. Blank entries are those events for which we could not find any relevant data. Key -<: Date represents age of youngest retina examined; event could begin before this date. >: Date represents age of oldest retina examined; event could continue after this date. N/A: event not applicable in this species. Numbers in brackets refer to the following citations. 1: Greiner and Weidman (1981); 2: Mey and Thanos (2000); 3: Robinson (1991); 4: Sernagor and Grzywacz (1995); 5: Reese *et al*. (1994); 6: Walsh and Polley (1985); 7: Reese and Colello (1992); 8: Snow and Robson (1994); 9: Drager (1985); 10: Henderson *et al*. (1988); 11: Williams *et al*. (1986); 12: Wong and Hughes (1987); 13: Potts *et al*. (1982); 14: Schmid and Guenther (1996); 15: Hughes and McLoon (1979); 16: Young (1984); 17: Schmid and Guenther (1999); 18: Wingate and Thompson (1995); 19: Maslim *et al*. (1986); 20: Pei and Rhodin (1970); 21: Hinds and Hinds (1974); 22: Greiner and Weidman (1982); 23: Linden *et al*. (1981); 24: Shatz (1983); 25: Bunt *et al*. (1983); 26: Godement *et al*. (1984); 27: Crabtree (1990); 28: Wong *et al*. (1993); 29: Meister *et al*. (1991); 30: Galli-Resta and Maffei (1988); 31: Catsicas *et al*. (1998); 32: Wong *et al*. (1998); 33: Sernagor *et al*. (2000); 34: Wong (1999a) and unpublished observations; 35: Zhou (1998); 36: Chapman and Stryker (1993); 37: Tootle (1993); 38: Masland (1977). Entries without a reference are unpublished observations by the authors (ES and ROLW).

the complex yet highly organized manner by which RGCs differentiate, establish their synaptic connections and begin the task of sensory coding.

2. STRUCTURAL DEVELOPMENT

RGCs are invariably amongst the first retinal cells to differentiate. Labeling of dividing cells with tritiated thymidine demonstrates that no other retinal cells are generated before RGCs and few RGCs are produced late in development (Polley *et al.*, 1989; Snow and Robson, 1994; Rapaport *et al.*, 1996; Belecky-Adams *et al.*, 1996; Cepko *et al.*, 1996). Although overlapping to some degree, the sequence of cell generation is similar across species: typically, after RGCs and then cones, the next cells to be born are horizontal and amacrine cells, followed by rods and bipolar cells, and finally by Müller cells (reviewed by Cepko *et al.*, 1996; Rapaport *et al.*, 1996).

Upon becoming postmitotic, RGCs migrate to the ganglion cell layer and begin the long process of structural and functional development (summarized in Fig. 2). RGCs extend an axon into the optic nerve even before elaborating dendrites (Morest, 1970; Hinds and Hinds, 1974; Maslim *et al.*, 1986). Rudimentary processes tipped with growth cones emerge from the cell body opposite to the axon initial segment, as the ventricularly directed process retracts (Maslim *et al.*, 1986; Kirby and Steineke, 1991, 1996). Soon after, a complex dendritic arbor takes shape with processes that ramify at different depths of the IPL. Synapse formation is first established with amacrine cells, and later with bipolar cells. With maturation, the dendritic arbor becomes confined to either the ON or the OFF sublamina of the IPL. In mammals, anatomical and functional refinement continues until several weeks after eye-opening, although the overall structural organization of the RGCs (Robinson, 1991) and their ability to generate ON and OFF responses to light are established by this stage (Masland, 1977; Rusoff and Dubin, 1977; Tootle, 1993).

2.1. Dendritic growth — overall patterns

Although the sequence of events underlying RGC differentiation as outlined in Fig. 2 is the same across the retina, the rate at which RGCs mature varies with retinal position (Rapaport *et al.*, 1996; reviewed by Robinson, 1991). Generally, for each major class of RGC, dendritic arbors attain their final organization and size more quickly in central retina compared to peripheral retina. Within a species, there are also differences in the rate at which the dendritic arbors of different RGC subclasses develop. For example, in the kitten, the dendritic arbors of alpha cells in the peripheral retina reach their adult dimensions at around three weeks after birth whereas beta cell trees change little in size until after the second postnatal week (Dann *et al.*, 1988). In contrast to alpha and beta RGCs, the arbors of gamma RGCs are adult-like at birth (Ault and Leventhal, 1994).

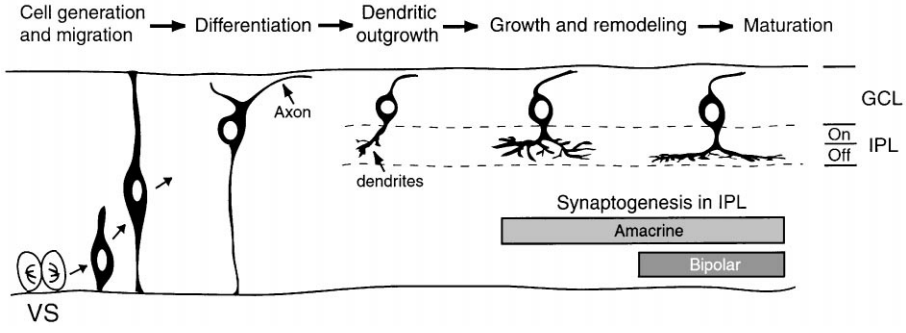

Fig. 2. Schematic representation of the major stages (left to right) in the development of a RGC. After becoming postmitotic and upon reaching the GCL, the RGC elaborates an axon and dendrites. As the dendritic arbor grows, it also reorganizes and eventually stratifies into the inner (ON) or outer (OFF) half of the IPL by maturity. Stratification takes place during the period of synapse formation with amacrine cells and bipolar cells. GCL, ganglion cell layer; IPL, inner plexiform layer; VS, ventricular surface.

Differences in the rate of maturation between major classes of RGCs are also observed in the rabbit retina; the dendritic trees of alpha cells continue to increase in area long after those of small and medium field RGCs reach their mature extent (Wong, 1990). Taken together, these observations suggest that the expansion of RGC dendritic arbors is unlikely to result solely from passive stretching of the retina (Mastronarde *et al.*, 1984). Maturation of each subclass of RGC may depend on a cell-specific growth program and/or include active interactions with the retinal environment and their central targets (see Section 2.3).

2.2. Dendritic remodeling — nature, dynamics and function

RGC dendrites do not grow in a straightforward manner but undergo much dynamic rearrangement, both in the addition and removal of existing processes. Such structural changes occur not only during the early period when the dendritic arbor grows but also during the period of synapse formation in the IPL. The loss of dendritic processes involves mainly small filopodium-like structures (terminal processes $<5\,\mu m$ long) that cover the dendrites of immature RGCs profusely (Dann *et al.*, 1988; Ramoa *et al.*, 1988; Wong, 1990). Like other CNS neurons, dendritic filopodia of RGCs are distinguished from classical dendritic spines by their size and morphology (Harris, 1999). Earlier reports have referred to RGC dendritic filopodia as spines, but these two profiles are not necessarily identical in structure or function (Harris, 1999). In contrast to other CNS neurons, only a few classes of RGCs bear spines at adulthood; in fact, most major classes of adult mammalian RGCs have "smooth" dendritic arbors (Dann *et al.*, 1988; Ramoa *et al.*, 1988; Wong, 1990).

Because dendritic filopodia are most prominent during the period of synaptogenesis in the IPL, it has been suggested that these transient profiles are involved in synapse formation (Wässle, 1988). Some insight into their potential synaptogenic function has recently been gained by watching their behavior in real time. Comparison of the dendritic arbors of RGCs labeled with green fluorescent protein (GFP) over several hours reveals a tremendous degree of reorganization involving the addition, elimination, extension and retraction of processes (Fig. 3). These changes

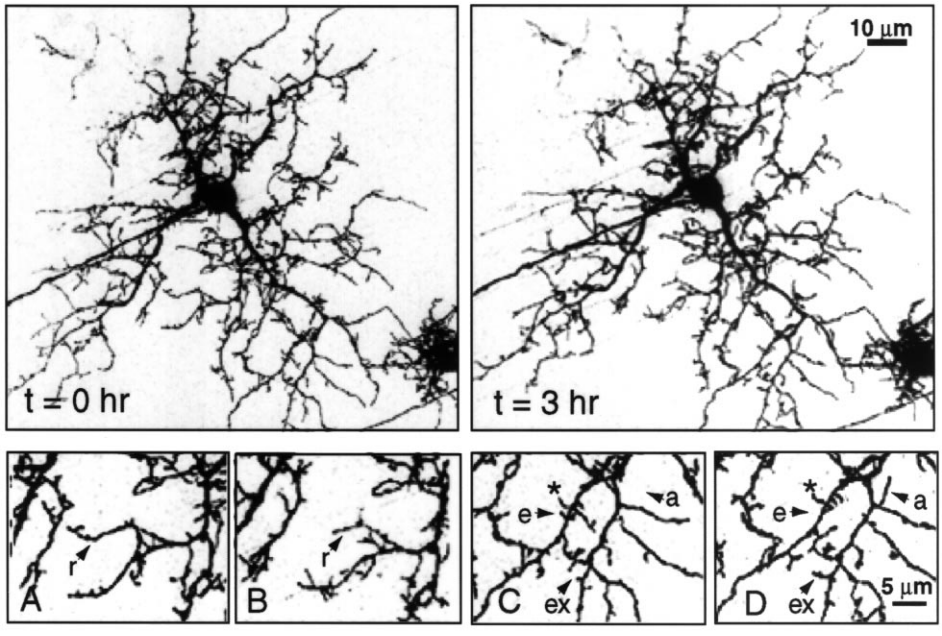

Fig. 3. Confocal images of an embryonic day 12 chick RGC labeled with green fluorescent protein. The top two images show the same RGC three hours apart. Structural changes are shown for two regions of the arbor (A–D). (B) and (D) are images of the regions (A) and (C) taken 3 h later, respectively. r, retraction; e, elimination; ex, extension; a, addition; asterisk, stable process.

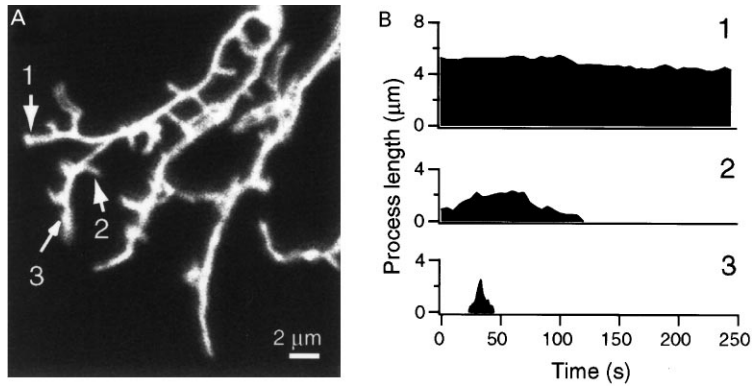

Fig. 4. Dendritic filopodia exhibit various structural behaviors. (A) Confocal image of an arbor from an embryonic day 13 chick RGC labeled with green fluorescent protein. The lengths of processes 1,2 and 3 are plotted over time in (B). While process 1 was stable during the length of recording, processes 2 and 3 had relatively short lifetimes.

largely involve dendritic filopodia which exhibit surprisingly rapid changes over the time scale of seconds to minutes. The rapid turnover of processes may help the dendrites of RGCs reach out more effectively for incoming afferent terminals of bipolar cells or amacrine cells. This is because, over time, the environment surrounding the dendrite would be "sampled" many fold more as a consequence of the rapid extension and retraction of filopodia. The time-lapse observations also provide an explanation for why at any one time (such as when tissue is fixed for electron microscopy) many dendritic filopodia are not associated with differentiated presynaptic terminals (Wong *et al.*, 1992). Their participation in synapse formation may therefore be one of initiating initial contact, whereupon a synapse can subsequently be formed nearby (see Harris, 1999).

Three other observations also implicate a synaptogenic role for dendritic filopodia. First, even though adjacent dendritic filopodia behave quite differently (Fig. 4), over many hours of recording, the number of processes that are added *de novo* appears balanced by the number of branches that are eliminated (Wong *et al.*, 2000b). This balance leads to a relatively constant total dendritic length and number of branch points suggesting that the rapid changes do not contribute primarily to growth within several hours. Second, the dendritic movements observed in developing RGCs are found to decrease with age, such that by the end of the period of synaptogenesis, the arbor is relatively stable over a period of hours (Wong *et al.*, 2000b). Third, blockade of neurotransmission inhibits filopodial motility, indicating that communication with presynaptic cells may be important (see Section 2.3.6). However, to determine whether RGC dendritic filopodia do in fact participate in synapse formation in the IPL, it will be necessary to watch this event occur between labeled pre- and postsynaptic processes (see Jontes *et al.*, 2000).

Apart from the gain and loss of small dendritic filopodia, in mammals, the number of branches (processes $> 5\,\mu m$ long) also initially increases and decreases with further maturation. Dendritic branches are pruned during the period of synaptogenesis (Dann *et al.*, 1987, 1988; Ramoa *et al.*, 1987, 1988; Wong, 1990; Yamasaki and Ramoa, 1993). Pruning is especially prominent in RGCs with relatively large dendritic arbors (such as alpha cells in the cat and rabbit retina), but is less dramatic in RGCs with relatively small dendritic trees (such as beta cells in cat and small-field RGCs in rabbit). In contrast to mammals, dendritic growth of RGCs in lower vertebrates such as goldfish appears to involve only the addition of material to existing processes (Hitchcock and Easter, 1986; Bloomfield and Hitchcock, 1991). However, recent work in embryonic turtle retina demonstrates that some dendritic processes of large-field RGCs are lost with age (Mehta and Sernagor, unpublished observations). This suggests that the elimination of a number of dendritic branches with maturation may be species-dependent or even cell-type-dependent, and that this

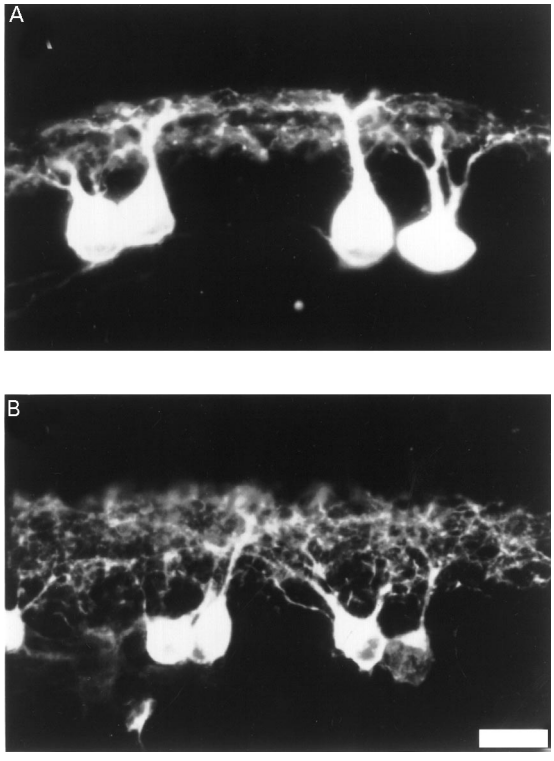

Fig. 5. Development of dendritic stratification in RGCs. (A) In the more mature retina (shown here for beta RGCs in the cat retina at postnatal day 10), the dendrites of RGCs occupy separate sublaminae (ON or OFF) within the inner plexiform layer (IPL). (B) By contrast, in the immature retina (postnatal day 2), the dendritic arbors are not stratified, but diffusely arranged within the IPL. Scale bar 20 μm. Photomicrographs kindly provided by L. M. Chalupa.

process is not unique to warm-blooded vertebrates such as mammals and marsupials (Dunlop, 1990).

It is evident that the loss of dendrites can contribute to establishing the final branching patterns of RGC dendritic trees. A likely consequence of this pruning is the emergence of ON and OFF stratification of RGC arbors in the IPL (Fig. 5). The normal progression of dendritic stratification has been examined in cat (Maslim and Stone, 1988; Bodnarenko *et al*., 1995), ferret (Bodnarenko *et al*., 1999) and primate (Kirby and Steineke, 1991). In all these species, dendritic processes initially span more than one sublamina in the IPL; some cells have bistratified arbors and others possess unstratified dendrites. Interestingly, within a local region of retina, the rate at which RGCs achieve a stratified arbor does not depend on cell class, and in fact not all neighboring RGCs attain their final dendritic organization at the same time (Maslim and Stone, 1988; Lohmann and Wong, unpublished observations). However, at any one age and region, both ON and OFF stratified arbors are present, indicating that ON and OFF stratification proceeds at the same time.

Although a stratified arbor appears to arise from the removal of ''misplaced'' dendrites, it cannot be deduced easily from histological examinations whether the loss involves major branches or only terminal processes. Furthermore, comparison of material fixed at different ages does not indicate whether stratification also includes the *addition* of new processes to the arbor within the appropriate lamina. Because small-field RGCs do not show a significant loss in their branch number during the period of ON–OFF stratification in the IPL, dendritic processes are likely to be added to balance those that are lost. One way to determine how RGC arbors become stratified is to carry out time-lapse imaging of this process in action. To date this has been difficult to achieve because dendritic stratification takes several days to weeks to accomplish, far too long for it to be possible to maintain RGCs in explant cultures. Co-culturing retinal explants with their normal central targets may provide a means to extend the survival time during which dendritic remodeling can be followed more extensively. A more likely solution would be to view dendritic development of GFP-expressing neurons *in vivo* in animals amenable to genetic manipulation and highly suitable for long-term imaging, such as Xenopus and Zebrafish (Jontes *et al*., 2000). Until then, our view of how ON–OFF stratification occurs in RGCs must rely on the piecing together of snapshots of this process over time.

2.3. Mechanisms regulating dendritic development

Dendritic development of RGCs involves (i) formation of dendritic branching patterns that are characteristic of different subclasses of RGCs, (ii) refinement of the dendritic arbor into ON and OFF strata, and (iii) formation and maintenance of neighbor relationships giving rise to the mosaic-like distribution of subtypes of cells across the

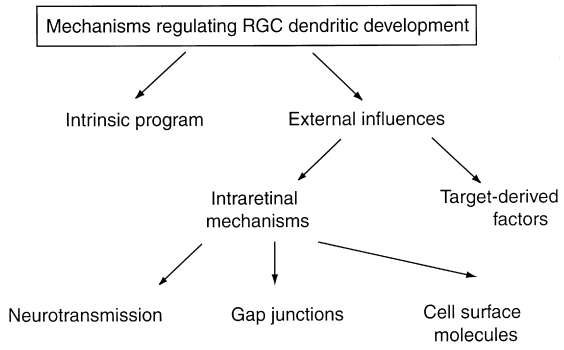

Fig. 6. Summary of potential mechanisms influencing RGC dendritic development.

retinal surface. Two broad categories of factors have been suggested to influence dendritic development of RGCs: factors that are intrinsic (genetically programmed) or extrinsic to the RGC. Extrinsic factors may arise from visual targets or within the retina itself. Retinal-derived factors may be diffusible or contact-mediated via synapses, gap junctions or adhesion molecules. A flow-chart summarizing these various factors that together influence RGC differentiation is provided in Fig. 6. The contribution of each of these factors is discussed next.

2.3.1. *Intrinsic programs*

Despite the diversity of dendritic branching patterns expressed by RGCs, cells with similar function within a retina bear a striking resemblance to each other. In addition, dendritic patterns of one subclass are reproducible from animal to animal. Together, these observations suggest that there may be inherent similarities, and differences, at the level of gene expression that regulate the morphologies of different subclasses of RGCs. Studies of dissociated neonatal cat RGCs support this hypothesis to some extent. In the absence of contact with neighboring RGCs and central targets, dissociated kitten RGCs are able to regenerate arbors similar to those observed in the retina *in vivo*, and distinct from cells in other parts of the CNS (Montague and Friedlander, 1989, 1991). In addition, several different types of dendritic morphologies are observed. Thus, the pattern of dendritic branching is likely to be controlled to some degree by mechanisms intrinsic to the cell. But, in these studies, the RGCs were isolated at an age when they had already developed distinctive morphologies. It is therefore possible that prior to this stage, environmental factors interact with intrinsic programs to determine some aspects of dendritic structure.

However, as will be discussed in the following sections, cell-type specific interactions between RGCs raise the possibility that RGCs of one subtype share common genetic components. Thus, there may be intrinsic differences between RGC subclasses or subtypes. New approaches in molecular biology involving the use of DNA microchips (Serafini, 1999) may help resolve the gene-profiles of morphologically or functionally classified RGCs.

2.3.2. *Target-dependent factors*

RGC survival depends on their forming contact with their targets (Sefton *et al.*, 1987; Aguayo *et al.*, 1996). However, it is less well known how target-derived factors help pattern the RGC dendritic arbor during development. In Xenopus, RGCs develop normal morphologies when the eyebuds are transplanted into a region distant from visual targets (Sakaguchi, 1989). This suggests that morphological differentiation of RGCs can occur independently of their central targets. However, work in the hamster visual system demonstrates that the branch complexity in RGCs can be influenced by their targets; a population of aberrant-projecting RGCs which normally simplify in their morphology with development fail to do so when their axonal environment is altered by monocular enucleation (Wingate and Thompson, 1994). The differences in the degree to which visual targets influence the final size and morphologies of RGCs may depend on when target support or other influences are removed during development, specifically, before or after axons have reached and made connections.

Target-derived factors are likely to include neurotrophins, which affect RGC axonal development and regeneration (von Bartheld, 1998; Cui *et al.*, 1998). Recent studies now demonstrate that neurotrophins also influence dendritic branching patterns of RGCs both *in vivo* (Lom and

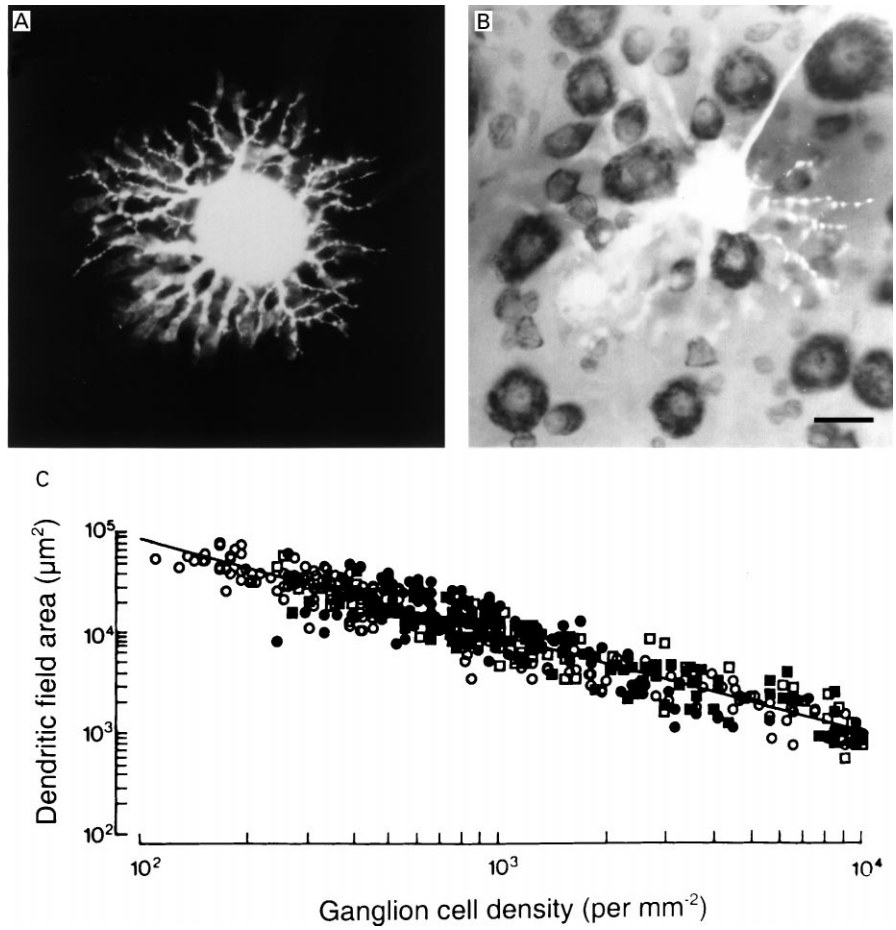

Fig. 7. A strict relationship between tree area and cell density is established at birth in many mammals. Shown here is an example of such a relationship for beta RGCs in the cat (R. O. L. Wong, D. I. Vaney and A. Hughes, unpublished observations). (A) Example of a beta RGC in the adult retina, dye-filled with Lucifer yellow. (B) The density of RGCs in the vicinity of a dye-filled RGC is obtained subsequently after Nissl staining. (C) Plot of dendritic tree area versus RGC density for P1 (filled squares); P10 (open squares); P21 (filled circles) and adult (open circles). Scale bar 10 μm.

Cohen-Cory, 1999) and *in vitro* (Bosco and Linden, 1999). Although the targets of RGC axons are a likely supply of such trophic factors, sources are also located within the eye (Lom and Cohen-Cory, 1999). With the escalating interest in neurotrophin signaling, we are likely to learn more about how these factors shape dendritic outgrowth, remodeling and maintenance in RGCs in the near future. One interesting aspect would be to determine whether activity-dependent refinement of RGC dendrites may involve neurotrophin signaling in a similar way that has been described for axonal refinement (Shatz, 1997).

2.3.3. *Neighbor interactions*

That interactions taking place within the retina influence the dendritic morphology of RGCs is implicit in the observation that the size of the dendritic tree varies in inverse proportion to local RGC density (Fig. 7; Rodieck, 1998). This close relationship between arbor size and cell density is apparent by birth in mammals (Fig. 7; Dann *et al.*, 1987, 1988; Wong, 1990).

The most striking observation demonstrating a role of the cellular environment in regulating the development of RGC dendritic arbors comes from

lesion studies in which a region of retina is completely depleted of RGCs after laser ablation (Eysel *et al.*, 1985) or axonal bisection (Linden and Perry, 1982; Perry and Linden, 1982; Leventhal *et al.*, 1989; Linden, 1993; Deplano *et al.*, 1999). In these animals, RGCs redirect their arbors toward the RGC-free zone, within which the inner retina remains intact. This response can only be evoked if the lesions are performed in early neonates, suggesting that mammalian RGC dendrites are able to reorganize only within a restricted period in development.

Uniform lowering of RGC density by target ablation also causes an expansion of the RGC dendritic field but not an exaggerated orientation in any one direction (Perry and Maffei, 1988; Bähr *et al.*, 1992). The effect of reducing RGC density on dendritic morphology and field size has also been examined in the chick retina by using clever manipulations to alter the density of cells without inducing cell death (Troilo *et al.*, 1996). Visual form deprivation results in a larger eye in which RGC density is reduced by 20–30%. In these retinae, the arbors of RGCs increase to match the areal change of the retina. In contrast, reduction of RGC density by partial optic nerve section produces somewhat distinct results — although RGC arbors are also found to increase in size, their branching density also increases. Changes in the complexity of branching are not observed in form-deprived retinae, suggesting that branching may be controlled by local interactions such as the availability of synaptic inputs, whereas the overall arbor size may depend on the spacing between RGCs and their neighbor relationships. Manipulations have also been performed to raise the density of RGCs during development. Monocular enucleation, which rescues some cells of the remaining eye from naturally occurring cell death, results in significantly smaller dendritic arbors by amounts proportional to the increase in cell density (Kirby and Chalupa, 1986; Leventhal *et al.*, 1989). Taken together, these studies indicate that the extent of the dendritic arbor is highly regulated by the local density of RGCs.

However, the effects of cell density may not be straightforward. At the border of the lesion, cells whose arbors reorient in the direction of the cell-sparse region have relatively fewer dendritic processes in the unlesioned zone compared to normal (Linden and Perry, 1982; Linden, 1993; Deplano *et al.*, 1999). This suggests that the overall distribution of dendritic processes may be kept "balanced" across the arbor, such that when one part of the arbor elaborates preferentially, other parts of the arbor withdraw. In addition, in the chick experiments (Troilo *et al.*, 1996), the ratio of the ON and OFF dendritic field areas of bistratified RGCs was also altered in the cell-reduced retina. This implies that mechanisms within the retina that regulate the size of RGC dendritic trees may act differentially on ON and OFF arbors (although it is possible that this effect is restricted to bistratified cells).

Further studies have asked the intriguing question of whether the dendritic arbor size of each class of RGC is regulated simply by local cell density or whether it is sensitive only to alterations within each RGC class. By deleting portions of the cat visual cortex at birth, beta cells, but not alpha cells, are selectively ablated from the retina (Ault *et al.*, 1993; Weber *et al.*, 1998). In the absence of beta cells, the area of alpha cell somas increased in central retina (and decreased in peripheral retina), leading Ault *et al.* (1993) to conclude that alpha cell development is influenced by both other alpha cells and beta cells. Similar results rejecting class-specific mechanisms were achieved when both alpha and beta cells were ablated in a local strip of retina (Deplano *et al.*, 1994). In contrast, the results of Weber *et al.* (1998) suggest that alpha cell development is not altered in cortex-ablated animals. The experimental factors that could contribute to the disparate findings include differences in the extent of the cortical lesions, and in the method of cell labeling. More direct assessment of whether or not dendritic development depends on cell-specific interactions may require ablation of a few, rather than the majority, of neighboring RGCs of one subclass (e.g. alpha or beta) or subtype (ON or OFF).

How does RGC density regulate dendritic elaboration? At least two mechanisms come to mind: diffusible factors such as growth factors, and specific signaling requiring cell contact. The assessment of diffusible factors on RGC dendritic development is not straightforward because of possible confounding effects on neuronal survival and neurite outgrowth. The latter mechanism,

contact-mediated communication, has been proposed to occur between RGCs, and may occur directly between RGCs or indirectly via their circuitry.

2.3.4. *Dendro-dendritic contact between RGCs*

The regularity at which retinal neurons are distributed into cell-type specific mosaics have long taunted investigators to elucidate the mechanisms that generate these spatial patterns (see Cook and Chalupa, 2000 for a recent review). A mosaic distribution of RGCs appears to be present upon differentiation, even before the cells have migrated to the forming ganglion cell layer (McCabe *et al.*, 1999). As dendrites of RGCs elaborate, the spacing between cells need to be adjusted and regulated to attain the nearest-neighbor relationships observed in the adult. For many years now, investigators have suggested a mechanism of "contact-inhibition" between RGCs that would regulate the spread of their dendritic arbors as the retina expands (Wässle and Boycott, 1991). Physical juxtaposition of the processes of RGCs is implicated by tracer-coupling between neighboring RGCs of the same subtype (e.g. alpha ON or alpha OFF; see Vaney, 1994). Such homologous coupling is present in the developing retina and may be a means by which RGCs can exchange signals (Penn *et al.*, 1994). However, amacrine cells are also tracer-coupled to RGCs, suggesting that the dendrites of RGCs may not form gapjunctions directly with each other. Indeed, gap junctions between RGCs have not been observed by electron microscopy (Freed and Sterling, 1988), but as yet, have not been ruled out.

Recent multiphoton reconstruction of the arbors of neighboring ferret alpha RGCs provide some support for the presence of dendro-dendritic contact between these cells during development. The dendrites of alpha RGCs form fascicles for tens of micrometers during neonatal development (Fig. 8). Such dendritic associations are found from the earliest age studied (around birth in the ferret) and only between alpha cells of the same center sign (ON or OFF). Contact via dendritic filopodia has also been observed (Fig. 8). Although dendro-dendritic contact occurs in subtypes of RGCs, whether contact-mediated inhibition takes place remains to be investigated (see Wässle and Boycott, 1991, for review). Furthermore, whether the dendrites of other classes of RGCs (for example, beta cells) touch each other has yet to be examined. Potential interactions may occur through homophilic binding of adhesion molecules such as cadherins, which are expressed by RGCs (Matsunaga *et al.*, 1988; Faulkner-Jones *et al.*, 1999; Honjo *et al.*, 2000). Other forms of contact-mediated interactions such as Notch–Delta signaling have been proposed for cortical neurons (Sestan *et al.*, 1999; Redmond *et al.*, 2000) and may also need to be considered for RGCs which use this form of signaling early in development (Rapaport and Dorsky, 1998).

The formation of dendritic fascicles between alpha RGCs of the same center sign may initially depend on whether cells of opposite center sign can recognize each other. If so, the failure of neighboring alpha cells of opposite sign to form fascicles implies that they avoid making contact. Alternatively, fasciculation may only take place because cells of the same subtype share a common set of recognition cues. The fact that dendritic fascicles form even before dendritic arbors stratify suggests that it is likely that the "fate" of ON and OFF RGCs is already specified before stratification takes place. However, as discussed later, the completion of stratification and the emergence of pure ON or OFF responses in major populations of RGCs is also dependent on interactions with the environment. Whether RGCs whose arbors fail to stratify with age (as a result of perturbing neurotransmission *in vivo*; see Section 2.3.6) demonstrate altered mosaic distributions is difficult to assess because we rely on this very property to study mosaic formation. Molecular markers for ON and OFF cells, assuming they exist, are really needed to help solve these issues.

2.3.5. *Axonal feedback from RGCs*

Another means by which RGCs may signal each other is via feedback through axon collaterals. Intraretinal axon collaterals have been observed in a variety of species in the mature animal (Dacey, 1985; Usai *et al.*, 1991). Electron microscopy reconstruction of the rabbit and cat retinal nerve fiber layers has also revealed synaptic feedback from RGC axons onto other axons and onto

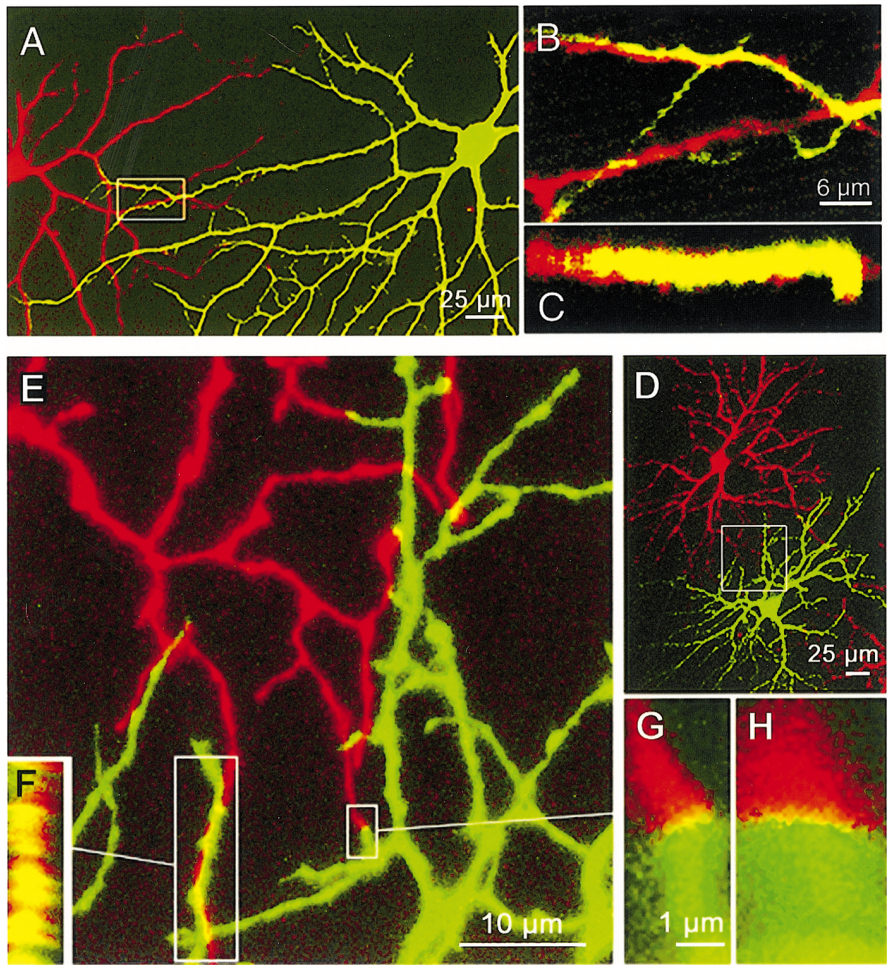

Fig. 8. Multiphoton imaging of pairs of alpha RGCs of the neonatal ferret retina, dye-filled either with sulfurhodamine 101 (red) or oregon-green (green). (A) Low-power magnification of a pair of dye-filled alpha cells at three weeks after birth. The boxed region is enlarged in (B); (C) is a 90 degree rotation of (B). Yellow pixels indicate regions where the dendrites of the two cells are in close contact. (D) The dendrites of neighboring ferret alpha RGCs at postnatal day 3. The boxed region is enlarged in (E). At this age, dendrites can contact each other via fascicles (left box; 90 degree rotation shown in F) or filopodia (right box; shown at higher magnification in G with 90 degree rotation in H).

displaced amacrine cells. Although lower in density compared to synaptic distributions in the inner and outer plexiform layers, synapses in the "superplexiform layer" (Wieniawa-Narkiewicz and Hughes, 1992) reveal a mode of communication that may need to be taken into account when considering how RGCs might interact with their neighbors.

Axon sprouts within the retina are certainly observed during development, but many of these disappear by maturity (Ramoa *et al.*, 1988). Although RGC axons commonly give rise to short sidebranches (Ramoa *et al.*, 1988), in some cells, axon collaterals are extensive and highly branched. Figure 9 is an example of an embryonic chick RGC with an elaborate axon collateral that terminates in the IPL. Whether these axon collaterals provide synaptic feedback within the IPL is currently unknown, but it would be an interesting issue to explore in the future. In particular, it will be useful to determine whether only specific subsets of RGCs exhibit extensive axon collaterals. More importantly, it remains to

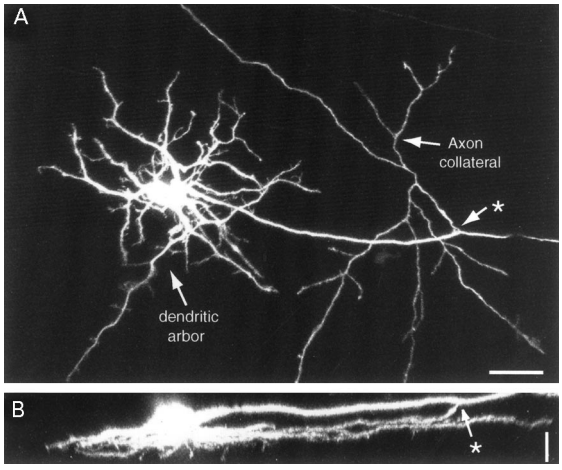

Fig. 9. Axon collateral from an embryonic day 12 chick RGC. (A) RGC labeled by biolistic delivery of GFP. (B) Ninety degree view of the 3D reconstruction of the confocal image stack. This collateral dives into the IPL and arborizes at the level of the dendritic arbor. Asterisk indicates point of origin of the axon collateral. Scale bars 25 μm.

be elucidated whether these intraretinal axons contribute to coordinating activity between neighboring RGCs during development and at maturity (see Section 3.2).

2.3.6. *Afferent signaling*

While it remains to be determined whether direct interactions between RGCs help organize their dendritic arbors, a role for afferent transmission is apparent. Although visual stimulation itself does not appear necessary for the normal development of RGC dendritic arbors (Lau *et al.*, 1990), neurotransmission appears to play a significant role. It has long been known that neurotransmitters such as acetylcholine affect neurite outgrowth of RGCs in culture (Lipton *et al.*, 1988). Recent studies show that blockade of cholinergic transmission *in vivo* results in RGCs with reduced total dendritic length and branch numbers (Fig. 10). Likewise, dendritic arbors of the embryonic chick retina simplify in the absence of glutamatergic transmission during the period of bipolar synaptogenesis (Fig. 10). Neurotransmission also affects dendritic filopodial motility and remodeling (Wong *et al.*, 2000b). The developmental decline in dendritic filopodia in one class of

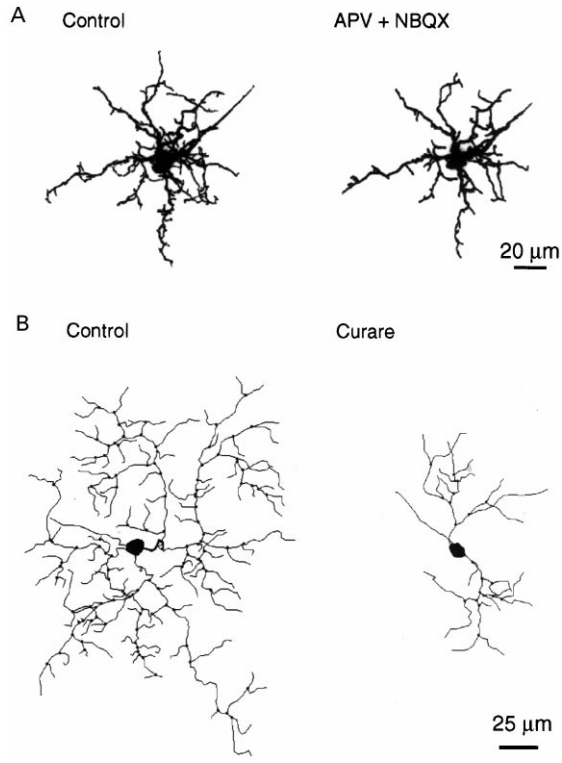

Fig. 10. Blockade of neurotransmission reduces dendritic arborization in RGCs. (A) Effects of blocking glutamatergic transmission on the dendritic arbor (labeled with green fluorescent protein) of a chick RGC *in vitro*. The arbor simplified after three hours of blockade with 100 μM APV and 10 μM NBQX. (B) *In vivo* chronic blockade of cholinergic transmission by curare (1 mM) in the embryonic turtle retina reduced the dendritic arborization (labeled with horseradish peroxidase) of RGCs.

RGC in the hamster is prevented by chronic blockade of NMDA receptors (Lau *et al.*, 1992). This may be because the motility of dendritic filopodia decreases in the absence of ionotropic glutamatergic receptor activation (Wong *et al.*, 2000b). Both the rate and extent of filopodial movements are reduced in the presence of antagonists to NMDA and non-NMDA receptors. These observations suggest that the production and dynamics of dendritic filopodia are regulated by neurotransmission, which is consistent with a role for these structures in facilitating synapse formation. RGC dendrites may respond to the secretion of neurotransmitter from incoming afferents either in a general trophic manner by putting out more filopodia, or in a directional manner by extending filopodia towards the stimulus. Interestingly,

transmitter regulation of dendritic motility is specific to the neurotransmitter utilized by the type of retinal interneuron making synaptic contact with RGCs. During the period of bipolar synaptogenesis, glutamate (the bipolar cell transmitter), but not acetylcholine or GABA (amacrine cell transmitters), affects dendritic motility in RGCs. The reverse is true during amacrine synaptogenesis (Wong and Wong, unpublished observations).

The neurotransmitters that regulate dendritic motility in RGCs during the period of synaptogenesis also serve to drive spontaneous bursting activity in these cells in a manner suitable for the reorganization of their axonal terminals (see Section 3.2). However, although important for the refinement of axonal structure and connectivity, action potential activity in RGCs is not necessary for the dynamic remodeling of their dendritic filopodia. The sodium channel blocker, tetrodotoxin (TTX), does not alter the nature and dynamics of dendritic filopodia movements (Wong *et al.*, 2000b). Intraocular injections of TTX into the kitten eye do not prevent the normal loss of dendritic filopodia with maturation (Wong *et al.*, 1991), and in fact result in a small increase in the number of dendritic filopodia (Wong *et al.*, 1991; Campbell *et al.*, 1997). However, chronic exposure to TTX does reduce the branch number of the dendritic arbor (Wong *et al.*, 1991) and also prevents the reorientation of RGC arbors into cell-free zones in lesioned retinae (Deplano *et al.*, 1994).

Neurotransmission is important for the developmental reorganization of RGC arbors into ON and OFF sublaminae of the IPL (Bodnarenko and Chalupa, 1993; Bodnarenko *et al.*, 1995). Chronic intraocular injections of DL-2-amino-4-phosphonobutyric acid (APB), a Group III metabotropic glutamate receptor agonist, perturbs the emergence of ON–OFF stratification in a reversible manner (Bodnarenko *et al.*, 1995). The site of action of APB in the immature retina is not entirely clear: although APB does not activate dissociated RGC somata (Liets and Chalupa, 1996), it may act through binding other Group III metabotropic glutamate receptors on RGCs (Duvoisin *et al.*, 1995). Whether bipolar cell transmission is necessary for the development of ON and OFF arbors is still unknown; to elucidate the role of such transmission, it will be necessary to block ionotropic glutamate receptors (NMDA and non-NMDA receptors) *in vivo*. If direct bipolar to RGC transmission is important, the segregation of arbors into ON and OFF laminae may be the result of competition between ON and OFF bipolar cells for synaptic targets. Recent studies by Wang *et al.* (1999b) demonstrate that during development, alpha and beta RGCs initially receive convergent ON and OFF inputs. Thus, APB application, which results in abnormal ON–OFF responses in RGCs (Bisti *et al.*, 1998), may prevent the normal segregation of ON and OFF inputs onto developing RGCs. The reorganization of ON and OFF inputs onto RGCs may require little restructuring of the bipolar axon terminals because these terminals are stratified even before ribbon synapses appear in the IPL (Miller *et al.*, 1999; Gunhan-Agar *et al.*, 2000). Interestingly, bipolar terminals are stratified even in the absence of RGCs (Gunhan-Agar *et al.*, 2000).

How could bipolar transmission sculpt the dendritic arbors of RGCs? As proposed by Bodnarenko *et al.* (1995), regions of the dendritic tree that receive little innervation by bipolar cells may eventually be lost whereas relatively well innervated parts of the arbor may be maintained and continue to elaborate. Support for this model comes from the observation that maintenance of RGC dendrites depends in part on glutamatergic signaling (Fig. 10). However, as yet, it is not known whether local blockade of glutamatergic transmission within parts of the arbor leads to selective elimination of the "less active" processes.

Several observations also suggest that amacrine cells may play a role in guiding the remodeling of RGC dendrites to form ON and OFF stratified arbors. First, RGCs begin stratifying before making synaptic contact with bipolar cells, but at a time when amacrine synapses have formed (Bodnarenko *et al.*, 1995, 1999; Lohmann and Wong, unpublished observations). Second, the processes of starburst cholinergic amacrine cells are stratified well before ON–OFF stratification proceeds in RGCs. Third, *in vivo* blockade of cholinergic transmission with curare results in relatively smaller dendritic arbors (Fig. 10).

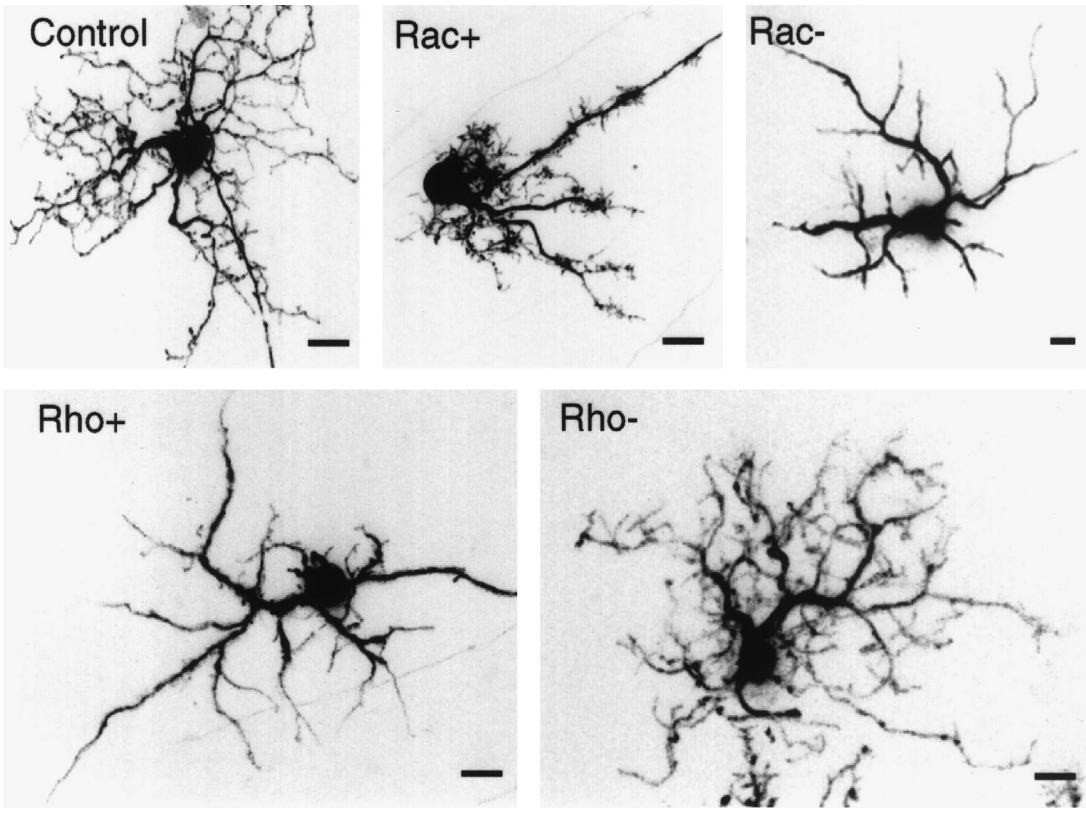

Fig. 11. Examples of the dendritic morphology of embryonic day 13 chick RGCs transfected with dominant negative (−) or constitutively-active (+) forms of Rac and Rho. These cells were visualized by cotransfection with green fluorescent protein. Scale bars 10 μm.

Finally, ON–OFF stratification in RGCs is perturbed in animals lacking the $\beta 2$ nicotinic receptor subunit (Bansal *et al.*, 2000).

2.3.7. *Intracellular signaling pathways*

The formation and remodeling of RGC dendrites involves the regulation of actin polymerization and depolymerization by the Rho family of small GTPases. The role of Rac1, RhoA and Cdc42 has been assessed by transfecting RGCs with dominant negative (dn) or constitutively active (ca) forms of these proteins. In the Xenopus embryo, Rac1, RhoA, and Cdc42 have distinct effects on dendritic and axonal development of RGCs (Ruchhoeft *et al.*, 1999). Dendritogenesis in RGCs requires Rac1 and Cdc42 activity. In contrast, axogenesis is inhibited by ca-Rac1. Over-expression of Rac1 also causes hyperproliferation of dendritic processes whereas ca-RhoA resulted in cells without dendrites. These observations parallel those observed in embryonic chick RGCs (Fig. 11). It is thus evident that across species, Rac and Rho have reciprocal effects on RGC dendritic structure — while Rac promotes the formation of new processes, Rho suppresses their appearance.

Time-lapse imaging of GFP-expressing RGCs transfected with dn- or ca-Rac and Rho implicates these molecules in regulating the dynamic changes in dendritic filopodial structure during the period of synaptogenesis. The movement of dendritic filopodia of cells transfected with dn-Rac and ca-Rho was significantly slower and less extensive than that of control cells (Wong *et al.*, 2000b). The challenge remains for us to determine how

extracellular signals, such as neurotransmission, regulate the levels of Rac, Rho and Cdc42 in ways that dynamically shape the dendritic arbors of the RGCs during development.

3. PHYSIOLOGICAL DEVELOPMENT

RGCs also undergo functional development and refinement concurrent with the growth and remodeling of their structure. Functional maturation of RGCs include the development of intrinsic membrane properties (the complement of ion channels and receptors), the formation and refinement of their circuitry, both within the retina and with their central targets, and the production of appropriate output signals. Although there is much interest in how RGCs develop their visual responses (receptive field properties), recent work has also raised the importance of studying spontaneous activity (non-visually evoked signals) in RGCs at ages when vision is not yet possible. This is because spontaneous neurotransmission in the immature retina is thought to be important for the refinement of RGC projection patterns which takes place before eye-opening (Goodman and Shatz, 1993; Wong, 1999a).

In this section, we will review what is known about the maturation of intrinsic membrane properties of vertebrate RGCs, the properties and function of patterned spontaneous action potential activity during development, and the subsequent maturation of their light response.

3.1. Intrinsic membrane properties

3.1.1. *Spike patterns of developing RGCs*

Ionic currents mediated by sodium, potassium, calcium and chloride act together to shape the excitability and spiking patterns of neurons. In the retina, sodium action potentials are generated only by RGCs and by some amacrine cells (Bloomfield, 1992, 1996; Cook and McReynolds, 1998) — the other major classes of retinal neurons, the photoreceptors, horizontal cells and bipolar cells, exhibit graded responses to light. Several major observations have prompted the investigation of developmental changes in RGC spontaneous firing patterns. First, such activity, and in particular, its spatiotemporal characteristics, is believed to be required for the developmental refinement of the connections between RGCs and their central targets (Goodman and Shatz, 1993; Wong, 1999a; see Section 3.2). Recent studies also implicate spontaneous neurotransmission in shaping physiological development of RGCs (Sernagor and Grzywacz, 1996; see Section 3.3). Second, all neurons in the CNS show an increase in excitability with development but little is known about the biophysical mechanisms underlying the maturation of firing behavior; RGCs are ideal and accessible models for such studies because the retinal circuitry remains intact and functional *in vitro*.

RGC excitability develops similarly across species. A significant proportion of RGCs are able to generate spikes in response to current injection during embryonic development, even before the retina becomes sensitive to light (examples: cat, Skaliora *et al.*, 1993; Robinson and Wang, 1998; mouse, Rothe *et al.*, 1999a; turtle, Mehta and Sernagor, unpublished observations). In mammals, RGCs are capable of spiking at the time their axons reach and contact their central targets during embryonic development, but before synapse formation commences within the retina at around birth. Not unexpectedly, an increasing proportion of RGCs are found to spike with maturation such that by eye-opening, all can generate action potentials (Robinson and Wang, 1998).

The major developmental change in firing behavior of RGCs is that they switch from single spiking to repetitive firing as they mature. In other words, the same sustained depolarizing current elicits only a single spike in immature RGCs, but evokes repetitive discharges in more mature cells (Fig. 12). In recent years, many studies have investigated the potential contribution of various conductances underlying the age-related changes in the spiking behavior of RGCs. To appreciate how developmental alterations in the expression or properties of these conductances could shape RGCs firing patterns, we will review what is known about their developmental regulation. Fig. 13 summarizes the up and down-regulation of Na^+, K^+ and Ca^{2+} conductances in several mammals. Taken together, the studies mainly show that the developmental changes in the firing

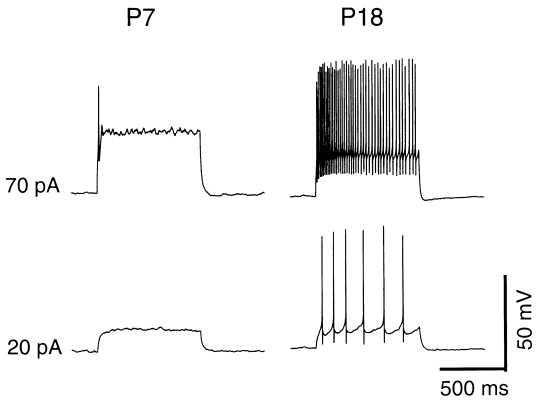

Fig. 12. RGCs demonstrate age-related changes in their excitability and spiking pattern. Relatively mature RGCs in the ferret retina (postnatal day 18, P18) can fire more spikes than younger RGCs (P7) in response to a fixed current step.

properties of RGCs are due to (i) increased Na^+ channel density and lowering of threshold for depolarization, (ii) changes in the voltage dependence and kinetics of Na^+ current activation or inactivation, and (iii) regulation by Ca^{2+}-dependent K^+ channels.

3.1.2. *Sodium currents*

Sodium conductances contribute to the firing threshold, amplitude and rate of rise of the action potential. Sodium currents (I_{Na}) slowly activate and then subsequently inactivate with depolarization relative to the membrane resting potential (V_{rest}). While the voltage-dependence of their activation controls the firing threshold, their inactivation properties determine the spike refractory period, and therefore influence the spike firing frequency.

Not surprisingly, the emergence of action potential discharges in RGCs and the subsequent decrease in firing threshold and increase in spike amplitude and firing frequency parallels increases in the expression of voltage-gated sodium channels. I_{Na} is present in RGCs before birth, and with subsequent development, its density increases and reaches adult values before or around the time of eye-opening (Fig. 13). Across species, the amplitude of I_{Na} also increases with maturation (see Robinson and Wang, 1998).

The activation and inactivation curves of I_{Na} alter with maturation, although the trends are not

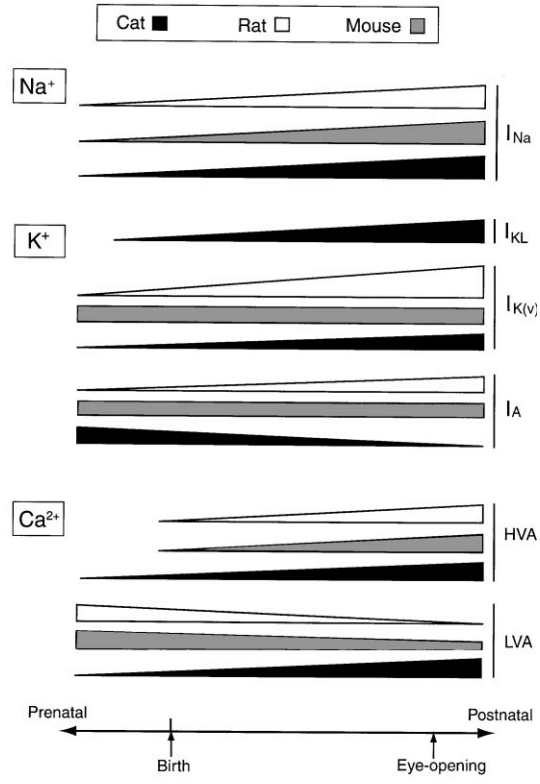

Fig. 13. Summary of the developmental changes of sodium, potassium and calcium conductances for some mammalian RGCs across development. For each conductance, a triangle or rectangle is drawn, shaded differently for each species. A triangle whose height increases or decreases to the right indicates that the current increases or decreases in density or amplitude during development; a rectangle indicates the current shows no significant change during development. I_{Na}, voltage gated sodium current; I_{KL}; a linear potassium current; $I_{K(V)}$, the delayed rectifier potassium current; I_A, a fast inactivating voltage-dependent potassium current; HVA; high-voltage-activated calcium current; See text for details. LVA; low-voltage-activated calcium current.

uniform across species. In cat (Skaliora *et al.*, 1993; Robinson and Wang, 1998) and rat (Schmid and Guenther, 1998), the I_{Na} activation curve shifts to more negative potentials whereas the inactivation curve shifts towards more positive potentials with maturation, enhancing the ability of RGCs to generate spikes. In mouse (Rothe *et al.*, 1999a), although the activation curve shifts to more negative values, there is no change in the inactivation curve. Because V_{rest} of RGCs also becomes more negative with maturation, the shift

in the activation curve towards more negative potentials with maturation does not necessarily imply that with age, RGCs more readily reach spike threshold. But, simultaneous measurements of I_{Na} and V_{rest} in neonatal ferret RGCs do show that for this species at least, a smaller depolarization step is required to elicit action potentials in relatively more mature RGCs (Myhr and Wong, unpublished observations).

The developmental increase in the density of sodium channels and a decrease in the action potential threshold together result in a greater ability of RGCs to generate spikes. Recent experiments also implicate the rate of recovery from sodium channel inactivation as a contributing factor to the emergence of repetitive firing in RGCs. The rate of recovery from sodium channel inactivation is slower in single spiking RGCs compared to those that exhibit repetitive spiking upon a sustained depolarization (Wang *et al.*, 1997).

3.1.3. *Potassium currents*

The generation and regulation of spike activity not only depends on sodium currents but also on potassium conductances acting in concert. Most importantly, potassium currents are involved in repolarizing cells after spiking, in reducing excitability, and in regulating spike shape and repetitive firing (Connor and Stevens, 1971; Lasater and Witkovsky, 1990). Many types of potassium currents have been characterized in RGCs according to their kinetics of activation and inactivation, and to their pharmacological sensitivity (see Ishida, 1995 for review). The major currents contributing to the total potassium current (I_{Ktotal}) are (i) I_A, a transient (fast inactivating) voltage-dependent current; (ii) $I_{K(V)}$, a voltage-dependent, gradually inactivating current (delayed-rectifier current); (iii) I_S, another voltage-dependent, slowly inactivating, current with different pharmacological sensitivity than $I_{K(V)}$, (iv) $I_{K(Ca)}$, calcium-dependent potassium current, triggered by the intracellular accumulation of calcium during electrical activity.

Potassium channel function is not only important for signal integration in mature neurons, but in development it also influences neuronal survival and gene expression by regulating neuronal excitability. In addition, K^+ currents help shape RGC firing patterns that could underlie synaptic refinement with their central targets (Wang *et al.*, 1998). These potential roles, taken together with the observations that RGCs develop different spiking patterns with age, have encouraged a more detailed examination of potassium channel function in RGCs during development.

The properties of various types of K^+ currents have been documented for several mammalian species across fetal and neonatal development. Generally, a diversity of K^+ currents are present even at early embryonic ages, when sodium currents appear. I_A and $I_{K(V)}$ are both present at fetal ages in cat, mouse and rat (Skaliora *et al.*, 1995; Rörig and Grantyn, 1994; Rothe *et al.*, 1999a; Guenther *et al.*, 1999; Reiff and Guenther, 1999). Other voltage-gated K^+ currents have been found during development: In cat, a linear K^+ conductance, I_{KL}, is present about three weeks before birth (Skaliora *et al.*, 1995). Another voltage-sensitive potassium conductance, I_B, is present in a small proportion (15%) of mouse RGCs towards the latter half of the first postnatal week (Rothe *et al.*, 1999a). In dissociated postnatal rat RGCs, some ganglion cells also demonstrate the slowly inactivating K^+ current, I_S (Sucher and Lipton, 1992; Guenther *et al.*, 1999; Reiff and Guenther, 1999).

Alterations in channel properties such as the kinetics and voltage dependency of activation and inactivation occur with maturation and therefore contribute to firing behavior (see Robinson and Wang, 1998). However, the most prominent change with age is the emergence of a pronounced sustained component in I_{Ktotal} by maturity. Such a change in the kinetics of total K^+ conductance could contribute to the increasing ability of RGCs to fire repetitively with maturation. The relatively slower inactivation of I_{Ktotal} at older ages has been attributed to a change in the relative balance of the transient (I_A) and the sustained-type (such as $I_{K(V)}$, I_B) components in rat RGCs (Reiff and Guenther, 1999), or the absence of I_A in some cells in the cat (Skaliora *et al.*, 1995). In mouse, however, there is no significant difference in the densities of I_A and $I_{K(V)}$ or in the fractional contribution of these currents to I_{Ktotal} between embryonic and postnatal stages (Rothe *et al.*, 1999a). These disparate

observations across species suggest that no one type of K^+ conductance underlies the emergence of the more sustained conductance with age. Furthermore, the ability to fire repetitively in cat RGCs is independent from the relative contribution of I_A and $I_{K(V)}$ to I_{Ktotal}, although RGCs lacking I_A have longer spike durations (Wang *et al.*, 1997).

The contribution of K^+ currents to lowering V_{rest} with age has also been explored. Studies in the rat suggest that there are no significant changes in somatic K^+ currents until the end of the first postnatal week, when V_{rest} has already reached mature levels (Guenther *et al.*, 1999; Reiff and Guenther, 1999). In cat, it has been suggested that I_{KL}, a linear K^+ current, contributes to V_{rest}. I_{KL} density, which increases with development, should help shift V_{rest} towards more negative values, approaching the K^+ equilibrium potential. The lowering of V_{rest} would also release Na^+ channels from inactivation, resulting in lowering the threshold for firing.

Ca^{2+}-dependent K^+ currents play a key role in the regulation of oscillatory behavior and spiking activity (Blatz and Magleby, 1987). Although this component of the K^+ current has not been investigated extensively in early development, their potential function in modulating spiking patterns in RGCs has been studied in the late postnatal ferret. Single channel recordings from dissociated postnatal ferret RGCs near the time of eye-opening (P30–46) show the presence of two types of Ca^{2+}-dependent K^+ channels: a large-conductance Ca^{2+}-dependent K^+ channel (BK_{Ca}), and a small-conductance Ca^{2+}-dependent K^+ channel (SK_{Ca}) (Wang *et al.*, 1998). Blockade of BK_{Ca} channels with charybdotoxin (CTX) or blockade of SK_{Ca} channels with apamin both lead to a decrease in the spike after hyperpolarization and to a shortening in the time to reach action potential threshold during current injection (Wang *et al.*, 1998). In addition, pharmacological blockade of the two types of Ca^{2+}-dependent K^+ conductances alters the spontaneous spiking patterns of neonatal ferret RGCs in two distinct ways. At the stage when RGCs exhibit spontaneous sustained firing, addition of CTX and apamin both induce burst-like activity (Wang *et al.*, 1999a). While in CTX, the bursts are brief, occur frequently, and are highly periodic; in apamin, the interburst interval is relatively long and the bursts are less regular (Wang *et al.*, 1999a). Apamin-induced bursts resemble the spontaneous bursts observed in more immature RGCs, suggesting that maturing SK_{Ca} conductances may be a factor involved in the switch from bursting to sustained firing in maturing RGCs (see Section 3.2).

3.1.4. *Calcium currents*

Studies in the developing retina have classified calcium currents in RGCs into three broad categories, according to their voltage dependence and kinetics of activation and inactivation. They are the transient low-voltage-activated (LVA; T-type) currents and the transient or sustained high-voltage-activated (HVA) currents. HVA conductances include those that are sensitive to ω-conotoxin GVIA (N-type), dihydropyridines (L-type) or insensitive to toxin altogether (Schmid and Guenther, 1996, 1999).

Just before birth, LVA-type currents dominate the calcium conductance in mouse and rat RGCs (Rörig and Grantyn, 1994; Schmid and Guenther, 1996; see Fig. 13). In rat, the number of cells exhibiting LVA conductances decreases with development and is zero by adulthood (Schmid and Guenther, 1999). Such a decrease in the density of LVA currents is also observed in the mouse (Rothe *et al.*, 1999a). By contrast, the density of LVA currents and the incidence of RGCs exhibiting this current increases with maturation in the cat (Huang and Robinson, 1998). From these observations, we may conclude that changes in the expression of LVA are not related to the development of repetitive firing in mouse and rat. In cat, the developmental increase of this current which activates at subthreshold potentials could enhance repetitive firing by reducing the time to threshold.

HVA currents are present in cat (Huang and Robinson, 1998; Robinson and Wang, 1998), mouse (Rörig and Grantyn, 1994; Rothe *et al.*, 1999a) and rat (Karschin and Lipton, 1989; Schmid and Guenther, 1996, 1999) RGCs from early embryonic stages. Their density increases with development, and they exhibit significant

changes in their activation and inactivation properties (Robinson and Wang, 1998). Together with LVA currents, transient HVA currents are more prominent in embryonic compared to postnatal cat RGCs, resulting in a larger number of channels available for activation at V_{rest}. As development proceeds, transient HVA currents in cat and sustained HVA currents in rat undergo a substantial hyperpolarizing shift in their activation curves. Sustained HVA currents in rat exhibit a developmental decrease in inactivation rate and in the steady-state inactivation time constant. In contrast, transient HVA current inactivation curves in cat shift during development towards more positive potentials. Together with an increase in Ca^{2+} channel density with age, the developmental changes in activation and inactivation kinetics of HVA conductances would result in an increase in the magnitude of Ca^{2+} influx per spike (Robinson and Wang, 1998).

What roles might Ca^{2+} channels play in RGC development? Ca^{2+} influx plays a key role in the regulation of many early developmental processes including cell survival and neurite outgrowth (McCleskey, 1994; Spitzer *et al.*, 1995). A recent study has shown that activation of Ca^{2+} channels in newborn rat RGCs influences dendritic remodeling (Heng *et al.*, 1999). Since the kinetics of HVA currents are slow, it is more likely that they will influence slow mechanisms such as repetitive firing and integration of synaptic responses rather than spike initiation. For instance, HVA channels have been found to mediate GABA-evoked Ca^{2+} influx in the embryonic chick retina, at a stage prior to neurite outgrowth and synaptogenesis (Yamashita and Fukuda, 1993). Later developmental events such as the emergence of repetitive firing may also be regulated by voltage-activated Ca^{2+} conductances through the activation of Ca^{2+}-sensitive K^+ conductances (Rothe *et al.*, 1999a). Furthermore, the development of repetitive firing features in RGCs is delayed in brain-derived neurotrophic factor (BDNF)-deficient mice (Rothe *et al.*, 1999b). This effect, which is attributed to a delayed increase in Na^+ channel density, is mimicked by Ca^{2+} channel blockers, suggesting that Ca^{2+} regulation may also influence the development of other cationic conductances.

3.2. Patterned spontaneous activity — retinal waves

In the mature visual system, RGC axonal projections are highly stereotypic. Projections from the left and right eye are separated, RGC subtypes (e.g. ON and OFF) innervate different target cells, and visual space is systematically represented as retinotopic maps. However, the initial patterns of connectivity are imprecise and undergo refinement during development through a process that is activity-dependent (reviewed by Goodman and Shatz, 1993; Crair, 1999; Penn and Shatz, 1999; Wong, 1999a). Synaptic transmission from the retina is required to fine-tune the early projection patterns of RGCs (Cramer and Sur, 1997, Penn *et al.*, 1998), and to maintain their mature configuration (Dubin *et al.*, 1986; Chapman, 2000). In mammals, because remodeling of RGC projection patterns takes place prior to maturation of photoreceptors, retinal activity responsible for such remodeling must occur independent of vision. In fact, many studies now demonstrate that even before the retina becomes light-sensitive, RGCs exhibit action potential activity with spatiotemporal characteristics that could guide the refinement of their axonal projections (Goodman and Shatz, 1993; Crair, 1999; Wong, 1999a).

3.2.1. *Spatiotemporal properties*

In vitro extracellular recordings of neonatal rabbit retina first revealed that developing RGCs periodically fire bursts of action potentials in the absence of light stimulation (Masland, 1977). In a technically challenging set of experiments, Galli and Maffei (1988) (see also Maffei and Galli-Resta, 1990) demonstrated that bursting activity is present *in vivo* in the fetal rat retina. Recordings from embryonic turtle further showed that RGCs exhibit a wide variety of spontaneous discharges throughout development (Grzywacz and Sernagor, 2000; see Fig. 14). At early stages, despite the frequent changes in membrane voltage, RGCs often fail to reach spike threshold. With maturation, spiking becomes more reliable, and burst durations lengthen (Fig. 14). In mammals, the discharge patterns of RGCs gradually switch from brief periodic bursts to more sustained firing with

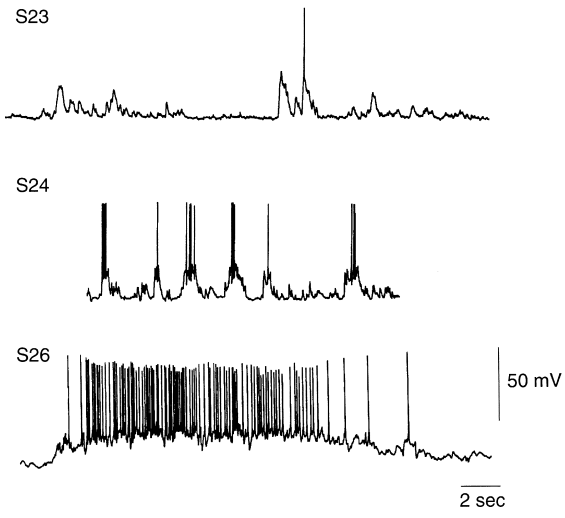

Fig. 14. Spontaneous activity in developing turtle RGCs. Whole-cell patch clamp recordings of spontaneous rhythmic activity under current clamp are shown at three different stages (S23–26). At the early stages, there are many subthreshold events that do not lead to spiking. Spiking ability and burst duration increase with age.

increasing age (Tootle, 1993; Wong *et al.*, 1993). Rhythmic bursting activity thus appears to be characteristic of the immature retina, and also of other parts of the nervous system such as the spinal cord and hippocampus (O'Donovan, 1999), suggesting that it may serve a fundamental role in development.

Recordings of Maffei and Galli-Resta (1990) additionally showed that the bursts of neighboring RGCs are temporally correlated. Later, simultaneous recordings from large populations of developing ferret and cat RGCs using a multielectrode array revealed that the bursting activity propagates across the retina as waves (Meister *et al.*, 1991; Wong *et al.*, 1993). Rhythmic bursting activity, recorded electrophysiologically or with calcium imaging, has been observed in the developing retina of many species including ferret (Meister *et al.*, 1991; Wong *et al.*, 1993, 1995; Feller *et al.*, 1996), turtle (Sernagor and Grzywacz, 1993, 1994, 1995), cat (Tootle, 1993), mouse (Mooney *et al.*, 1996; Bansal *et al.*, 2000; Stellwagen *et al.*, 1999), chick (Catsicas *et al.*, 1998; Wong *et al.*, 1998; Sernagor *et al.*, 2000) and rabbit (Zhou, 1998). Propagating waves have been observed in several species to date (ferret, cat, rabbit, chick, mouse and turtle; see Fig. 15), and so are likely to be a general phenomenon.

Retinal waves can originate at any point in the retina and then propagate in random directions and patterns (Meister *et al.*, 1991; Wong *et al.*, 1993; Feller *et al.*, 1996). In ferret, waves spread within spatial "domains" whose boundaries alter with time, implying that there are no circuits unique to any region of the retina that give rise to this activity (Feller *et al.*, 1996, 1997). By contrast, waves in the chick (Wong *et al.*, 1998; Sernagor *et al.*, 2000), mouse and turtle are extensive, propagating across millimeters of retina and often terminating at the retinal edges (Fig. 15; but see Bansal *et al.*, 2000). Waves tend not to invade regions that have just been activated (Feller *et al.*, 1996, 1997; Wong *et al.*, 1998). How frequently a wave passes a local region of retina, however, can vary from less than a second (Sernagor *et al.*, 2000; Fig. 16) to over a minute (e.g. Meister *et al.*, 1991; Wong *et al.*, 1993; Feller *et al.*, 1996).

What clues may be present in the correlated activity of RGCs, and in particular in the retinal waves, that could guide the refinement of the axonal projection patterns? Synaptic connections are thought to be refined according to the Hebbian postulate that synchronously active inputs will connect to the same postsynaptic cell, and conversely asynchronous inputs will connect to different postsynaptic cells. Both theoretical models and experiments based upon blockade of retinal activity suggest that retinal waves provide cues to refine at least two aspects of RGC axonal projections. First, neighboring RGCs within an eye tend to fire together within a wave, providing enough information to make a smooth retinotopic mapping of visual space in the retinal targets (Simon *et al.*, 1992; Eglen, 1999). Second, waves are independently generated in each eye, and so RGCs from different eyes tend not to fire at the same time, driving eye-specific segregation in the LGN: each postsynaptic cell ultimately receives inputs from only one eye (Shatz and Stryker, 1988; Penn *et al.*, 1994; Eglen, 1999). From a theoretical viewpoint, the frequency of waves should not affect ocular segregation since the activity in each eye is still independent. However, if one eye generates waves more often than the other, LGN cells will be preferentially wired to the more-active

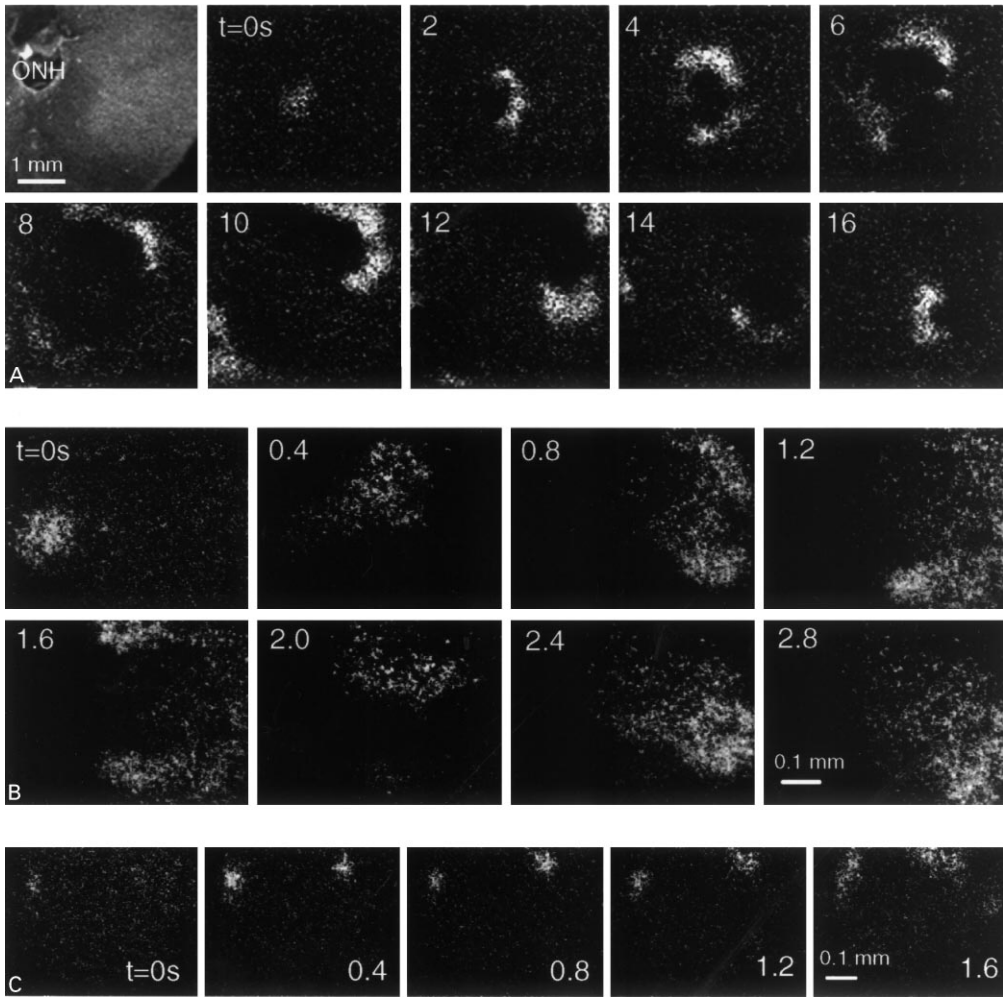

Fig. 15. Examples of waves propagating across a new born mouse retina (A) and embryonic turtle retina (B,C) observed by calcium imaging. Bright pixels represent an increase in activity. (A) The fura-2 labeled retina is shown in the top left image; subsequent difference images, acquired 2 seconds apart, show the propagation of activity. Difference images (160 ms apart) of activity in ganglion cells from a (B) Stage 23 (S23, about 2–3 weeks before hatching) and a (C) S26 (hatching stage) turtle retina. Cells were back-filled through the optic nerve with calcium green dextran. Movies of these waves are available on the internet at: http://neurosci.ncl.ac.uk/neurobiology/research/movies/retinalwaves.

eye (Eglen, 1999). The effect of wave velocity upon development has not yet been investigated theoretically or experimentally, although we suggest that changes in velocity will affect the correlations between neighboring cells which could in turn influence the refinement of retinotopic maps (Sernagor et al., 2000).

Recent experimental evidence now suggests that spontaneous activity may also guide the development of RGC dendrites as well as their axons (see Sections 2.2 and 3.3).

3.2.2. *Mechanisms that underlie and regulate patterned spontaneous activity*

Because of the functional importance of spontaneous retinal activity in establishing precise patterns of connectivity between RGCs and their central targets, investigators have sought to understand how rhythmic bursting is generated in RGCs, and what mechanisms lead to the propagation of activity across the retina. Determining the mechanisms may enable perturbation of the

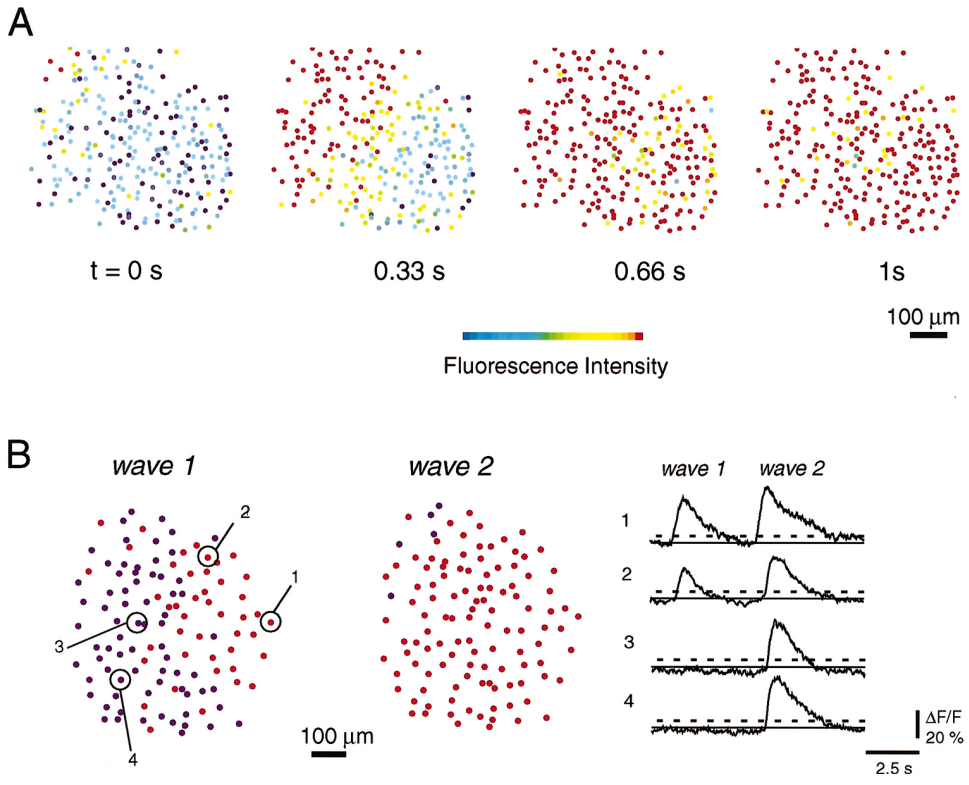

Fig. 16. Retinal waves in embryonic day 15 chick retina. (A) Example wave recorded at high temporal resolution (30 frames/second). RGCs were back-labeled from the optic nerve with calcium green dextran; each dot represents a labeled cell. Fluorescence level of each cell is color-coded from blue (baseline) to red (above-threshold). (B) Recruitment of cells during two consecutive waves, just seconds apart. Each cell is colored red if its fluorescence intensity exceeded the threshold during a wave, otherwise it is colored blue. Wave 1 recruited mostly cells on the right side of the field of view whereas wave 2 involved almost all cells. The fluorescence changes recorded in four representative cells during the two waves are shown in plots to the right (solid horizontal line is the baseline activity level, dotted horizontal line is the threshold).

spatiotemporal properties of the activity to be carried out *in vivo*. Taken together with modeling studies (Eglen, 1999), such perturbation studies should provide a better understanding of what information is conveyed by the retinal activity to drive synaptic refinement in their targets.

Synaptic transmission, presumably from retinal interneurons, is necessary for the generation of spontaneous bursting activity in developing RGCs (Sernagor and Grzywacz, 1993, 1999; Feller *et al.*, 1996). Propagation of action potential activity also ceases in the absence of synaptic transmission (waves measured using the multielectrode array disappear in cobalt; Wong, unpublished observations). In the absence of action potentials (such as in TTX), RGCs fail to spike, but integration of calcium signals within large regions of retina shows that rhythmic oscillations persist, although at a lower frequency and amplitude (Stellwagen *et al.*, 1999). However, no calcium rise is detected in individual RGCs when recordings are performed at high magnification (Wong *et al.*, 1995). Thus, rhythmic calcium fluctuations in TTX observed under low magnification (Stellwagen *et al.*, 1999) may have revealed activity in nonspiking retinal interneurons, such as some amacrine cells (Zhou, 1998), rather than in RGCs. Patch clamp recordings from turtle retina also demonstrate the presence of recurring subthreshold episodes of activity (presumably synaptic responses) in early embryonic turtle RGCs (Fig. 14) suggesting that spiking in RGCs itself is not essential for generating their spontaneous rhythmic activity. Taken together with direct measurements of activity from amacrine cells (Wong *et al.*, 1995; Butts *et al.*, 1999; Zhou, 1998), these calcium

imaging and patch recordings show that oscillations in activity also occur at the level of the cells presynaptic to the RGCs.

Two major types of excitatory neurotransmitter (glutamate and acetylcholine) drive rhythmic spontaneous burst activity in the RGCs (Wong, 1999a). Their relative contribution to the generation and regulation of the bursting activity, however, is age-dependent. At a stage when RGCs receive only amacrine cell input (Fig. 17), cholinergic (nicotinic) transmission is necessary (Sernagor and Grzywacz, 1996, 1999; Feller *et al.*, 1996). GABAergic transmission is also present at this stage and provides additional excitatory drive (Feller *et al.*, 1996; Fischer *et al.*, 1998), although such transmission appears not to be required for bursting to occur. In addition, in the rabbit, muscarinic transmission becomes important when nicotinic transmission is no longer required (Zhou and Zhao, 1999). The source of acetylcholine is likely to be the starburst amacrine cells, the only cholinergic cell in the retina (Masland and Tauchi, 1986; Vaney, 1990). When bipolar inputs develop later (Fig. 17), glutamatergic transmission becomes progressively more important for the generation of spontaneous bursting activity (Wong *et al.*, 1998; Sernagor and Grzywacz, 1999; Sernagor *et al.*, 2000; Wong *et al.*, 2000a). In turtle and ferret, the contribution of glutamate to spontaneous bursting activity is largely mediated by AMPA/kainate receptors rather than by NMDA receptors (Sernagor and Grzywacz, 1999; Wong *et al.*, 2000a), while in chick it appears to be mediated equally by both receptor subtypes (Wong *et al.*, 1998; Sernagor *et al.*, 2000). Glutamate is presumed to be secreted by bipolar cells but other sources are possible. For example, RGCs, which are also glutamatergic, may signal to amacrine cells or other RGCs via axon collaterals that terminate in the IPL (see Fig. 9; Dacey, 1985; Peterson and Dacey, 1998). Another possible source is that of immature rods and cones which transiently project to the IPL (Johnson *et al.*, 1999). The degree of overlap in the contribution of cholinergic (nicotinic) and glutamatergic transmission in driving spontaneous bursting of RGCs varies somewhat across species. For instance, while in turtle, both transmitters contribute at all times (Sernagor and Grzywacz, 1999), in rabbit,

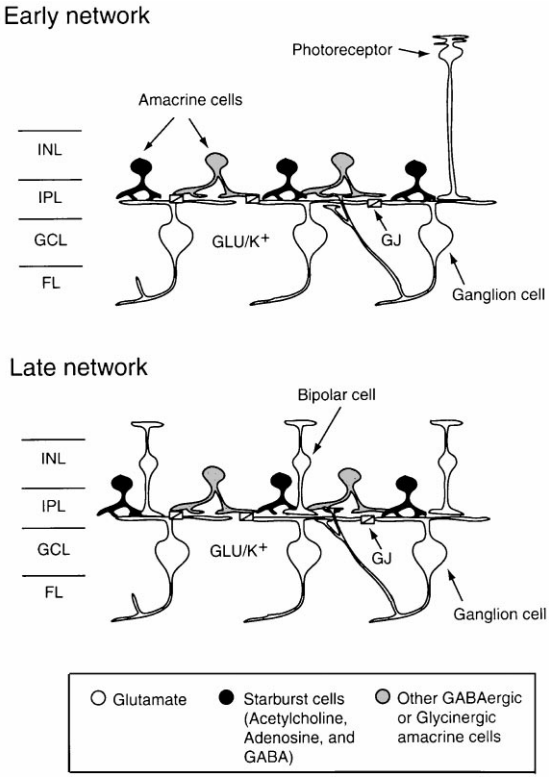

Fig. 17. A summary of possible cellular mechanisms underlying the generation of spontaneous bursting activity and wave propagation at two different stages of development. Glutamatergic cells and cholinergic amacrine cells provide the excitation necessary to generate bursting and are involved in wave propagation. Glutamate could originate from several sources: feedback from RGCs, inputs from bipolar cells and immature photoreceptors. Tonic release of extracellular glutamate (GLU) may also be important. Propagation of waves may also be mediated by gap junctions (GJ) involving ganglion cells and amacrine cells. Elevation in extracellular $K+$ upon depolarization of cells may also contribute to wave propagation. GABAergic and glycinergic amacrine cells modulate burst frequency and amplitude but are not involved in activity propagation. FL, fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer.

these forms of transmission regulate spontaneous bursting activity at distinct periods (Zhou and Zhao, 1999).

How do these various forms of neurotransmission contribute to wave generation and propagation? Despite the fact that interactions between RGCs and retinal interneurons are important, it is not clear how amacrine cells, bipolar cells (or undifferentiated photoreceptors), and RGCs are

interconnected to form a network that sustains retinal waves. Even less is known about how cellular components within the network act together to shape the spatiotemporal properties of the waves across development. However, both theoretical and experimental work is beginning to shape our basic understanding of how waves may be generated and propagate.

Using computer modeling approaches, Feller *et al.* (1997) suggested that uncorrelated spontaneous activity in the amacrine cells spreads laterally to other amacrine cells and converges onto neighboring RGCs to produce correlated waves. The identities of these amacrine cells are yet unknown, and although cholinergic transmission is necessary, cholinergic amacrine cells may not be the sole initiators of the waves because these amacrine cells also receive synaptic drive (Zhou, 1998). Other theoretical studies suggest that extracellular K^+, released during spontaneous firing, is involved in the propagation of waves (Burgi and Grzywacz, 1994a, b). This is supported in part by experiments in which K^+ channels are blocked (Sernagor and Grzywacz, 1999). However, the observation that not all neighboring neurons participate in a wave (Wong *et al.*, 1995) suggests that more complicated mechanisms, including synaptic connections, are involved.

Pharmacological studies dissecting the contribution of the various types of neurotransmission provide some insight into the mechanisms underlying wave propagation. First, cholinergic transmission is necessary for the propagation of retinal waves at early developmental stages (Feller *et al.*, 1996; Sernagor *et al.*, 2000). At later times, when glutamatergic inputs are inserted into the circuit, glutamatergic transmission regulates the speed of the waves but is not necessary for wave propagation (Sernagor *et al.*, 2000). Cross-correlation analysis of spontaneous bursting activity recorded from pairs of turtle RGCs reveals two components: an early, asymmetric and brief component, and a slow, symmetric component. The fast component may reflect feedback from one RGC to the other, or a common but stronger input to one RGC. The slow component may reflect propagation of activity which can occur from different directions (Sernagor and Grzywacz, 1999). Partial glutamate blockade abolishes the early component, while leaving the late component of the function unchanged, suggesting that glutamate may indeed be involved in local excitability but not regulate propagation *per se*.

Gap junctions represent an attractive mechanism for mediating the propagation of the waves throughout development. Before synaptogenesis, gap junctions are involved in the propagation of activity not only across the ganglion cell layer but also across the depth of the retina (Catsicas *et al.*, 1998). After synaptogenesis in the IPL, pharmacological blockade of gap junctions suppresses spontaneous activity in chick retina (Wong *et al.*, 1998), suggesting they play a role. Furthermore, tracer-coupling studies in the developing ferret retina indicate that alpha and gamma RGCs demonstrate homologous coupling (Penn *et al.*, 1994). However, gap junctions cannot solely propagate spontaneous activity, since no tracer coupling is found between beta cells, yet beta cells also fire during waves (Wong *et al.*, 1993; Wong and Oakley, 1996). Direct assessment of the contribution of gap junctions to wave propagation must wait until more specific gap-junctional blockers become available. Future experiments will require the role of gap junctions to be distinguished from the contribution of synaptic transmission and the extracellular diffusion of excitable agents.

What mechanisms regulate the temporal and spatial properties of the spontaneous activity in immature RGCs? Recordings of the burst patterns of individual RGCs suggest that there is a positive correlation between the burst duration and the preceding interburst interval — following a relatively long burst interval, cells burst for longer (O'Donovan, 1999; Grzywacz and Sernagor, 2000). The interburst interval represents some "refractory" state during which the RGC does not fire action potentials. The duration of the refractory period may be set by mechanisms intrinsic or extrinsic to the RGCs. Because RGCs are capable of generating spikes during the interburst interval when stimulated exogenously (Feller *et al.*, 1996), the refractory period may reflect the properties of the presynaptic cells or their interconnections, and of their inputs onto RGCs. Although regulation of burst frequency of individual cells could be important in itself

(control of mean firing rate), it is the synchronization of the bursts of neighboring cells that is likely to be the crucial factor for refining connectivity between the retina and its central targets (Goodman and Shatz, 1993).

Understanding the mechanisms underlying activity propagation will allow investigators to change the spatiotemporal properties of waves to see if the pattern of activity can guide the refinement of the axonal projections of RGCs. For example, waves that spread too quickly will synchronize the activity of more distant cells — a result that could lower the precision in the fine-tuning of retinotopic maps (Wong, 1999b). How then are waves regulated? Exclusion of waves from a local region of retina immediately after a wave has passed through are also likely to be regulated by mechanisms extrinsic to the RGCs. Modeling studies (Feller *et al.*, 1997) proposed that refractory periods of waves could be caused in turn by refractory periods in the presynaptic amacrine cells which might drive the spontaneous activity in RGCs. Recent experiments show that neuromodulators secreted by amacrine cells do in fact regulate the spatial properties of the waves. In the presence of elevated adenosine, waves sweep more frequently across a local region of retina, as well as increase in size (Stellwagen *et al.*, 1999). Dopamine, however, while applied *in vitro* can alter wave frequency, has no effect *in situ* (Stellwagen *et al.*, 1999). Blockade of GABAergic transmission appears not to affect the spatiotemporal properties of the waves at early stages (Stellwagen *et al.*, 1999).

Despite having established some key players involved in wave generation and propagation, much remains to be elucidated before we understand how retinal waves come about. This is because we have yet to unravel the exact circuitry that underlies this activity. For instance, although cholinergic amacrine cells are interconnected via synapses (Millar and Morgan, 1987; Millar *et al.*, 1987), not all these cells possess nicotinic receptors (Keyser *et al.*, 1988) or respond to nicotine (Baldridge, 1995). Yet, nicotinic receptor antagonists arrest wave propagation at early stages. Perhaps even more puzzling is the mechanism by which subsets of RGCs, not known to be interconnected, become synchronized in their bursting. For example, the activity of ON and OFF RGCs remains synchronized, even when their dendritic arbors are confined to different sublaminae within the IPL. Anatomical links between the ON and OFF pathways, such as via the AII amacrine cell (Vaney, 1990), are present, but they do not relay excitation across these two pathways. Clearly, much work is still needed in order to unravel the circuitry of the developing retina and in particular to determine the anatomical substrate for generating and sustaining waves.

3.2.3. *Age-related changes in the patterns of activity*

Several functionally important changes in the spontaneous activity patterns of RGCs occur with maturation. Initially, waves propagate across the retina, covering relatively large regions over time. With maturation, the activity becomes more localized to relatively small patches within which cells fire in near synchrony. These patches occur randomly in time and location. The change in the spatial arrangement of spontaneous activity with maturation is shown for the developing turtle retina in Fig. 15B,C. In ferret, multielectrode recordings indicate that waves disappear just before eye-opening, but local synchrony between neighboring cells has only occasionally been observed over the limited sampling area (0.5 mm diameter) (Wong *et al.*, 1993). The mechanisms that restrict lateral propagation of the activity are still unknown; the answers will be fundamental to our understanding of how synchronous firing between retinal neurons occurs and how it disappears.

Apart from changes in the spatial patterns of activity, there are also age-related alterations in the temporal patterns of activity. In ferrets, after eye-specific segregation is complete, ON and OFF RGCs develop distinct temporal patterns of spontaneous activity as their axonal terminals separate to occupy distinct sublaminae within the dLGN (Fig. 18; Wong and Oakley, 1996). During this period of development, although their bursts remain synchronized, ON RGCs adopt a much lower burst frequency compared to OFF RGCs. The emergence of disparate ON and OFF RGC bursting rhythms with age results in a difference in their mean firing rate and the degree of correlation

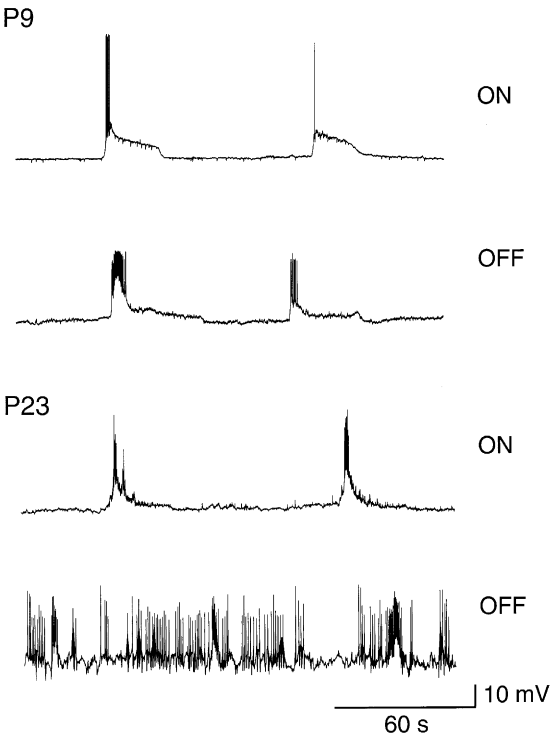

Fig. 18. Spontaneous activity patterns of developing ON and OFF neonatal ferret RGCs. Current clamp recordings at two ages (postnatal days (P) 9 and 23) indicate that ON and OFF RGCs develop distinct spontaneous spiking patterns with maturation. Recordings were obtained for each cell separately. At P9, ON and OFF RGCs are connected to amacrine cells (see Fig. 17, early network), and at P23, bipolar cell input is also present (late network).

in their activity. Computer modeling (Lee and Wong, 1996) suggests that such patterns provide information sufficient for the activity-dependent segregation of ON–OFF inputs to the LGN (Hahm *et al.*, 1991).

How do different ON–OFF rhythms arise with age? GABA becomes inhibitory at the time when ON and OFF rhythms diversify; and GABAergic transmission more effectively suppresses bursting activity in ON RGCs compared to OFF RGCs (Fischer *et al.*, 1998). However, the differential inhibition of GABA of ON compared to OFF RGCs is not sufficient to account for their different mean firing rates. In turtle, GABAergic neurotransmission modulates spontaneous bursting by shortening the duration of the bursts and reducing the firing frequency during bursting, but has no effect on burst frequency (Sernagor and Grzywacz, 1999). Although differences in synaptic circuitry may contribute to the age-related changes in ON and OFF bursting patterns, such changes could also originate from intrinsic membrane properties. Amongst many possibilities, mature OFF cells may develop a more prominent SK_{Ca} than ON cells (see Section 3.1; Wang *et al.*, 1999a), or OFF cells may exhibit a faster recovery from inactivation of Na^+ conductances compared to ON cells. (Wang *et al.*, 1997). The relative contributions of the changing network versus maturation of intrinsic membrane properties in shaping the temporal firing patterns of RGCs remains to be determined.

If retinal waves persist in mature animals, they would interfere with patterned vision. Interestingly, waves in mammals do disappear as RGCs become responsive to light (Wong *et al.*, 1993). Why? Although no experiments have addressed this issue as yet, some insight may be gained from two situations in which rhythmic bursting activity has been assessed in mature animals. First, in taurine-deficient cats, RGCs located within a patch of retina depleted of photoreceptors demonstrate rhythmic bursting activity that is uncharacteristic of mature RGCs, but resemble the pattern in immature cells (W. R. Levick, personal communication). Thus, the anatomical circuitry underlying rhythmic bursting activity, and perhaps waves, in RGCs may not be disassembled at maturity but rather masked by the emergence of the vertical photoreceptor pathway. Second, in the absence of visual experience, spontaneous bursting activity in RGCs persists for longer periods posthatching (Sernagor and Grzywacz, 1996), suggesting that early visual experience may trigger the disappearance of rhythmic bursting activity in these cells. How maturation of photoreceptors and visual experience terminate the occurrence of rhythmic bursting activity or waves is yet to be elucidated.

3.3. Light responses

Light-driven activity emerges as the vertical photoreceptor-bipolar pathway matures shortly before eye-opening (P10 for rabbit; Masland,

1977; Dacheux and Miller, 1981a, b; P7–10 for cat; Tootle, 1993). At this stage of development, the lateral connections to RGCs from amacrine cells are already well-established (McArdle *et al.*, 1977; Dacheux and Miller, 1981a, b; Maslim and Stone, 1986). Because it is likely that the basic circuitry underlying the receptive field organization of RGCs is present prior to when RGCs can respond to light stimuli, it has been difficult to investigate how connections develop to give rise to specific receptive field properties before vision. Furthermore, although visual stimulation in the isolated retina or in eyecup preparations is possible for the immature retina, the poor quality of the optics in neonates prevents reliable assessment of light responses *in vivo* (Thorn *et al.*, 1976). Nevertheless, important knowledge concerning the development of light responses and the underlying circuitry responsible for their generation has been gained using several electrophysiological approaches.

An overall assessment of the development of light responses is possible from electroretinograms. In rabbit, a small negative response (a-wave, reflecting photoreceptor activity) is present from P6, tripling in amplitude by P9–10 (Masland, 1977). From P10, the time of eye opening, there is also a small positive component (b-wave, reflecting K^+ uptake by Müller cells) in the response. Both a- and b-wave subsequently increase in amplitude and attain their mature profile a few weeks later (Reuter, 1976).

Direct measurements from RGCs show several major trends in the maturation of their light responses. The initial responses of RGCs to light stimulation are weak, labile and adapt rapidly (around P8 in rabbit — Masland, 1977; around P3–4 in cat — Tootle, 1993). Once robust responses to light become detectable a few days later, several adult features of RGC receptive fields are already apparent. Both in cat and in rabbit, the earliest measurable receptive fields are already concentric in their center-surround organization (Bowe-Anders *et al.*, 1975; Masland, 1977; Tootle, 1993); ON- and OFF-center receptive fields are also present. In the cat, there are no significant developmental changes in the proportion of cells with ON- or OFF-center receptive fields or in the percentage of cells with ON–OFF RF centers (Tootle, 1993). However, recent studies in the ferret retina suggest that prior to eye-opening, a larger proportion of RGCs receive convergent ON and OFF inputs (Wang *et al.*, 1999b). Other specialized features of RGC receptive fields, such as direction selectivity, are also apparent before eye-opening (Masland, 1977).

The maturation of the surround organization also appears to vary with species. In the rabbit, before eye-opening, there are large "undifferentiated" fields with silent surrounds that can suppress the response to center stimulation but do not themselves respond to direct light stimulation (Masland, 1977). In the cat, the strength of the antagonistic surround relative to that of the center does not seem to change with postnatal maturation. Silent inhibitory surrounds, however, are not observed until the third postnatal week in cat (Tootle, 1993).

How do the developmental changes in receptive field properties pertain to the acuity of RGCs? Recordings from kittens aged P5 and P35 suggest that the grating acuity of peripheral RGCs does not change during this developmental period, despite a decrease in area of the receptive field center (Tootle, 1993). Examination of the RGC density within the area centralis, however, suggests that grating acuity would increase after birth. This is because the density of central beta RGCs is adult-like at this stage and maintained throughout eye growth as the retinal magnification factor decreases with age (Wong and Hughes, unpublished observations).

3.3.1. *Mechanisms regulating receptive field development*

The development and rearrangement of connectivity in cortical and subcortical visual areas is known to depend on spontaneous retinal activity and visual experience (Wong, 1999a). However, less is understood about the role of activity or other mechanisms that influence the development of RGC receptive fields. In mammals, visual experience does not appear to affect the development of receptive fields of RGCs (Daw and Wyatt, 1974). When rabbits, shortly after eye-opening, were raised in an environment with unidirectionally moving stimuli, ON or OFF responses and

directional selectivity developed normally. Because rabbit RGC receptive fields are fairly mature by P10–15 (Masland, 1977), it is possible that visual experience had no impact on retinal organization from these ages. However, even prior to eye-opening, the major receptive field properties such as concentric center-surround organization and direction selectivity are already apparent. It is thus unlikely that the development of these characteristics relies strongly on visual experience, although the role of activity *per se* remains possible.

In recent years, the developing turtle retina has proven to be an ideal model for studying receptive field development in RGCs. RGCs in the turtle respond to light robustly during embryogenesis. They become driven by light from Stage 23 (S23, corresponding to about 3 weeks prior to hatching within a 60 day-long gestational period), almost coinciding with the onset of spontaneous bursting activity in these cells (S22) (Sernagor and Grzywacz, 1995). Immature turtle RGCs respond to light earlier than mammalian RGCs, although their receptive fields still change considerably during development. The receptive fields are initially small and continue to expand until 2–4 weeks post-hatching, when they reach their mature sizes. Strikingly, RGCs at the early stages respond well to several directions of movement or orientation of the stimulus bar, in contrast to maturity, when they prefer only one direction of motion or orientation, or have no preference at all (Sernagor and Grzywacz, 1995).

Theoretical studies have suggested that the response to multiple directions or orientations of motion in immature turtle RGC receptive fields is due to polarized and poorly branched dendritic arbors (Burgi and Grzywacz, 1997, 1998). Concomitant intracellular dye labeling of RGCs and mapping of their receptive fields do indeed indicate relatively good matching between the shapes of the dendritic and receptive fields in some S25 embryonic turtle RGCs (Fig. 19). However, dendritic arbors of other embryonic turtle RGCs are very large, branched and elaborate, even at S24 (Mehta and Sernagor, 1999), at a time when the cells can still respond to multiple directions of motion (Sernagor and Grzywacz, 1995). Thus, these responses to multiple directions of movement may not always be predicted from the shape of the dendritic arbor. Instead, responses to multiple directions may arise from immature sets of inputs whose nature (excitation versus inhibition) or distribution has yet to be fine-tuned. For example, a moving bar may evoke spiking in an immature RGC only if it activates all its presynaptic inputs simultaneously, rather than sequentially. Future experiments to correlate receptive field properties with other important factors, such as synaptic distributions onto RGC dendrites, are needed to further elucidate the contribution of both dendritic layout and the spatial distribution of synapses underlying their varied responses to light stimuli.

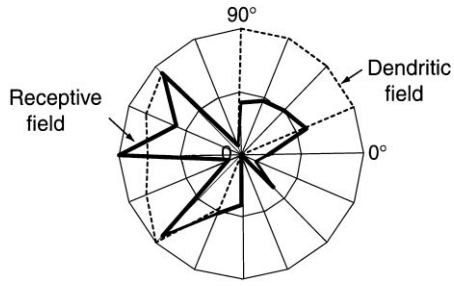

Fig. 19. Structure-function correlate of an immature turtle RGC. The bold trace depicts the normalized synaptic responses of a Stage 25 RGC to an edge of light moving at 16 equally-spaced directions of motion (at a speed of 4 degrees per second). The dotted trace shows the normalized maximal dendritic length of this cell measured at each corresponding angle. The correlation coefficient between the two measurements is 0.63.

Several experimental manipulations that modify spontaneous activity *in vivo* affect the development of receptive field properties in turtle RGCs (Fig. 20). First, dark-rearing of turtle hatchlings prolongs spontaneous activity and results in larger receptive fields (Sernagor and Grzywacz, 1996). In contrast, when cholinergic transmission is blocked from embryonic stages by *in vivo* application of curare, receptive field areas are relatively smaller. In fact, after 1 month posthatching, receptive fields of RGCs in curare-treated animals remain at sizes corresponding to those at the stage upon which the drug was applied (Fig. 20). Furthermore, exposure to curare from the day of hatching prevents the dark-induced expansion of receptive fields (Sernagor and Grzywacz, 1996). Taken together, these observations suggest that cholinergic-mediated spontaneous activity, but not visual stimulation,

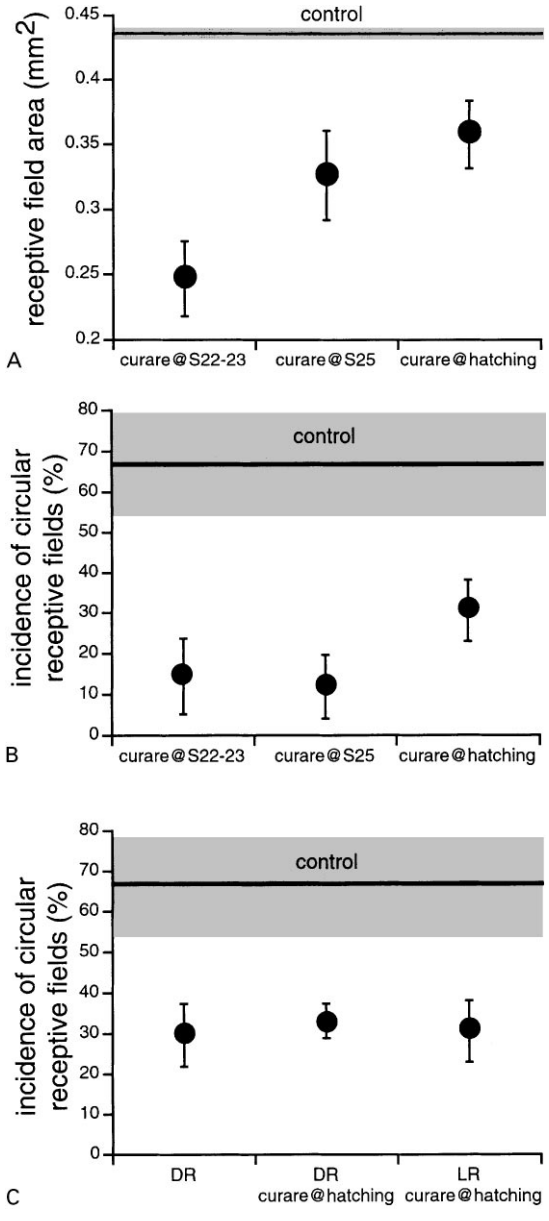

Fig. 20. The effects of spontaneous bursting activity on developing receptive fields. Control values are shown as a horizontal line across each plot, with the shaded grey area indicating the standard error of the mean. (A) Receptive field areas (with standard error bars) measured at about one month post-hatching when the retinas had been exposed to curare (1 mM) from different embryonic stages. (B) Same as (A), but for incidence of cells with circular receptive fields. (C) Incidence of circular receptive fields in dark-reared (DR) turtles with or without exposure to curare and light-reared (LR) turtles with exposure to curare.

is important for the development of receptive field area.

These experimental manipulations not only affect receptive field properties, but they also alter the underlying dendritic arbors of RGCs. Dark-rearing causes an increase in branch number and length, whereas curare-treatment results in fewer, and shorter, branches (Mehta and Sernagor, 1999). Furthermore, compared to normal animals, synaptic density in the inner plexiform layer is increased by dark-rearing, whereas it is decreased by curare-treatment (de Juan, Sernagor, Guardiola and Grzywacz, unpublished results). These structural changes may explain, in part, the enhancement and reduction in receptive field size in dark-reared and curare-treated animals, respectively.

The proportion of RGCs that have circular receptive fields increases with maturation (Sernagor and Grzywacz, 1995). Both curare-treatment and dark-rearing reduce the proportion of cells with circular receptive fields (Fig. 20). However, the incidence of RGCs with circular receptive fields in dark-reared turtles was similar to that of either dark or light-reared turtles whose retinae had been exposed to curare from hatching (Fig. 20). These observations indicate that both spontaneous bursting activity and visual stimulation contribute to the emergence of circular receptive field profiles.

How could spontaneous activity regulate the development of receptive field properties in RGCs? One possibility is that neurotransmission from bipolar and amacrine cells is sufficient, and patterned activity, such as waves, is not necessary. The other possibility is that there is information encoded by the waves that helps configure the appropriate receptive field properties in RGCs. To distinguish between these possibilities, it is necessary to design experiments in which spontaneous activity is maintained, but its spatio-temporal dynamics are altered. Both recent pharmacological experiments in which wave properties such as speed or width of the wavefront are altered (Sernagor *et al.*, 2000), and modeling approaches will hopefully provide a better understanding of how spontaneous neurotransmission helps set up RGC receptive fields during development.

4. SUMMARY

In the last decade, the development of structure and function of retinal ganglion cells has continued to be intensely studied. On the structural side, we know that RGC dendrites do not grow in a simple fashion, but instead undergo rapid remodeling. We also know some of the intracellular signaling pathways involved in such restructuring. Here we have highlighted several external influences likely to be involved, in addition to intrinsic influences, in controlling dendritic development. On the functional side, much work has gone into understanding the membrane properties associated with the development of spiking activity in developing RGCs. In addition, correlated firing patterns in the developing retina have been extensively investigated because of their potential role in the refinement of visual connections. Several mechanisms have now been discovered that could underlie the coordination of spikes into retinal waves. Finally, knowledge of these mechanisms has allowed experimenters to perturb patterned activity to investigate how activity can regulate the development of receptive fields and the underlying dendritic structure.

However, these findings still leave us with many questions unanswered. Currently, we can describe many extrinsic influences upon dendritic development, but their relative importance is still unclear. One limitation to date has been inferring developmental processes just by examining fixed tissue at different points in time. The recent improvements in live-imaging techniques mean that experimenters can now follow dendritic development over much longer periods. Also, we know little about the various intrinsic mechanisms that are likely to be important, for example, in generating the different classes of RGCs. However, with the recent ever-increasing advances in molecular techniques, we expect these tools will help us to discover the molecular differences between separate functional classes of RGCs. The close relationship between the structural and functional characteristics of RGCs means that these neurons will continue to be a valuable system for studying neural development.

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