

E. Sernagor and M.H. Hennig (2013). **Retinal Waves: Underlying Cellular Mechanisms and Theoretical Considerations**. In: Comprehensive Developmental Neuroscience: Cellular Migration and Formation of Neuronal Connections, J. Rubenstein, P. Rakic, eds, San Diego: Academic Press.

This is an uncorrected preprint, the final version is here:

<http://dx.doi.org/10.1016/B978-0-12-397266-8.00151-4>

1. Introduction

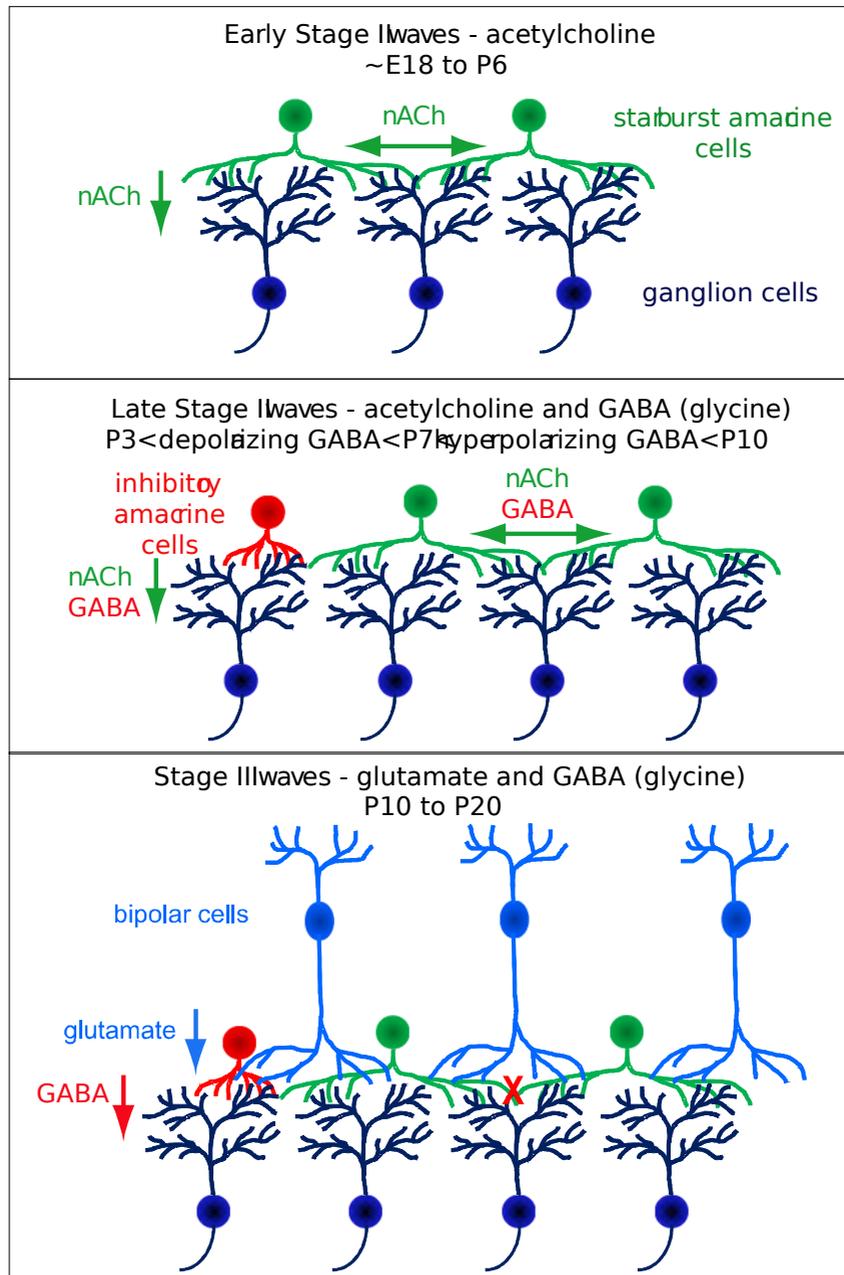
Long before visual experience is even possible, the developing vertebrate retina is already far from silent. *In vitro* extracellular recordings from the neonatal rabbit retina first revealed that developing retinal ganglion cells (RGCs) periodically fire bursts of action potentials in the absence of light stimulation (Masland, R. H. 1977). Subsequently, Galli, L. and Maffei, L. (1988) (see also Maffei, L. and Galli-Resta, L. 1990) succeeded in demonstrating that spontaneous bursting activity is present *in vivo* in the fetal rat retina. In mammals, these discharge patterns gradually switch from brief periodic bursts to more sustained firing with increasing age (Tootle, J. S. 1993; Wong, R. O. L. *et al.* 1993). In comparison, RGCs in turtle exhibit a wide variety of spontaneous discharges throughout development (Sernagor, E. and Grzywacz, N. M. 1995; Grzywacz, N. M. and Sernagor, E. 2000; Sernagor, E. *et al.* 2001).

This early spontaneous bursting activity consists of intense episodes lasting up to about 30 seconds, followed by quiescent periods lasting up to several minutes. These spontaneous bouts of activity emerge at some point during gestation and they disappear during the perinatal period. Remarkably similar activity patterns have been observed in many species, ranging from reptiles to monkeys, suggesting a common role for this early activity during the development of the visual system (Katz, L. C. & Shatz, C. J. 1996). Rhythmic bursting activity has been recorded in other parts of the developing nervous system (O'Donovan, M. J. 1999; Feller, M. B. 1999; Blankenship, A. G. and Feller, M. B. 2010), suggesting that such activity may serve a fundamental role in neural development.

The major hallmark of early retinal spontaneous activity is that it is correlated between neighboring neurons, resulting in propagating waves. This chapter introduces both experimental observations and theoretical aspects of the cellular mechanisms underlying the generation and propagation of retinal waves during development, discussing the similarities and differences between different species. Except for very early waves that can propagate indiscriminately across retinal layers, retinal waves are restricted to the inner retina, where, at early stages, they are generated in the inner plexiform layer (IPL) through cholinergic connections between amacrine cells (ACs) and retinal ganglion cells (RGCs), and at later stages through glutamatergic connections, presumably between bipolar cells (BCs) and ACs and RGCs (see figure 1).

Retinal waves are believed to play a key role in activity-dependent processes of visual development before visual experience is possible. Such processes include, for example, the development of retinal receptive fields (Sernagor, E. et al. 2001), the segregation into eye-specific layers in the dorsal lateral geniculate nucleus of the thalamus or the refinement of topographic maps in retinal projections (Wong, R. O. L. 1999; Torborg, C. L. and Feller, M. B. 2005). However, the goal of this chapter is not to discuss how retinal waves guide the wiring of the visual system, but rather to review how the spatiotemporal properties of the waves change with development. Such changes provide important clues about how retinal waves can indeed instruct the development of connectivity in the visual system.

Before plunging into the cellular mechanisms responsible for their generation, it is interesting to note that developing fishes and amphibians do not have spontaneous retinal waves. In the evolutionary scale, these waves appear only in reptiles and are preserved in birds and mammals. This surprising observation suggests that retinal waves do indeed play an important role in guiding the wiring of visual connections before the onset of visual experience. Both fish and amphibian embryos are translucent; hence spontaneous activity appears redundant because these species can use visual experience from early developmental stages for wiring visual connections. This is obviously not the case for reptiles or birds who develop under opaque egg shells (with the eggs often buried in the ground for protection against predators) and even less so for viviparous mammals.

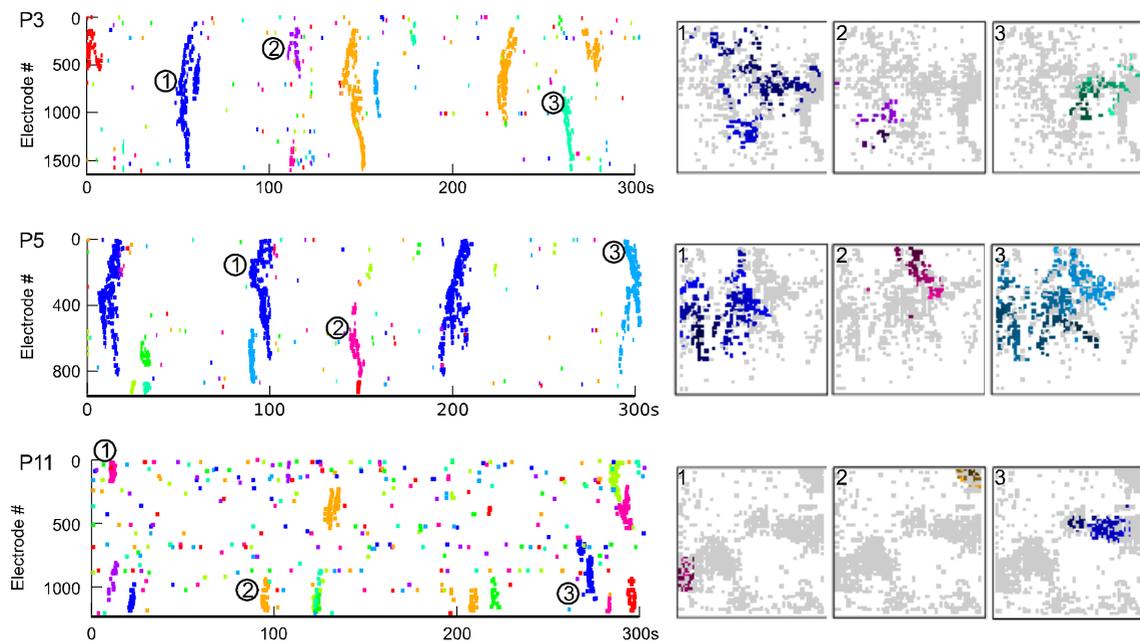


2. How do we record retinal waves?

Research on retinal waves necessitates simultaneous visualization from large cell assemblies. Retinal waves were discovered by recording with a multielectrode array (MEA) consisting of 64 extracellular electrodes in an 8x8 configuration (Meister, M. et al. 1991). Isolated retinas are flat mounted on the array with the RGC layer facing down on the electrodes; action potentials are recorded from RGCs in direct contact with the electrodes. Another approach to record retinal waves is cellular imaging. Calcium imaging is widely used for that purpose. Retinas are either labeled with a membrane permeant indicator (e.g. Wong, R. O. L. et al. 1995) that indiscriminately labels all cells (RGCs, displaced ACs) or with a membrane impermeant indicator that is applied through the optic nerve, thereby labeling exclusively RGCs (e.g. Sernagor, E. et al.

2000). Both MEAs and imaging have advantages and limitations. MEAs have the advantage of providing high temporal resolution as the electrodes continuously record changes in voltage, limited only by the sampling rate of the system. However, MEAs have serious limitations in terms of spatial resolution because the recordings are restricted to the area above the electrodes. Thus, if the separation between electrodes is, for example, 200 μm , this will limit the spatial resolution to that distance and events that do not propagate over that distance will not be detected. In addition, the separation of events from different neurons recorded with the same electrode is often difficult, limiting the analysis of the activity of neurons in isolation. Imaging has much better spatial resolution as in principle, it could label virtually every cell exposed to the dye, and each neuron's activity could be unambiguously monitored. However, cell loading becomes more problematic with retinal maturation. In mouse, for example, membrane permeant dyes do not load the retina properly beyond postnatal day (P) 4 and the indicator must be injected below the inner limiting membrane (e.g. Blankenship, A. G. et al. 2009). Another limitation of imaging is the poor temporal resolution. Calcium signals are slow and only indicative of neural activity; they cannot detect single spikes, or even very short bursts. Moreover, in many studies images were acquired both at low magnification (hence activity could not be discriminated in individual cells) and at low sampling rate (0.5-2 Hz). Fine grained spatiotemporal activity features cannot be detected at such low resolution. However, some studies using calcium imaging have used higher sampling rate (25-30 Hz) and looked at fluorescence changes in individual cells (Sernagor, E. et al. 2000; Sernagor, E. et al. 2003). These studies provide more accurate measurements of the spatiotemporal features of the activity in the developing retina. Another limitation of imaging is that calcium indicators bleach relatively quickly once exposed to light at their excitation wavelength. Therefore, imaging cannot be performed continuously over extended periods. The advent of genetically encoded activity indicators may overcome these limitations. Movie 1 shows an example of retinal waves recorded at P8 from a transgenic mouse expressing the fluorescent calcium indicator protein GCaMP2 (Diez-Garcia, J. et al. 2007) (with permission from E. Sernagor, H. Mutoh and T. Knöpfel).

With either approach, only a limited window of the retina can be sampled at any given time, and wave dynamics have been mostly extrapolated from these spatially restricted windows. Recent developments in MEA technology can now provide significantly better spatiotemporal resolution. The Active Pixel Sensor (APS) MEA is a powerful novel system consisting of 4,096 electrodes in a 64x64 configuration (Berdondini, L. et al. 2009) covering an area of 2.67x2.67mm, which encompasses virtually the entire neonatal mouse retina. The electrodes have a diameter of 21 μm and they are directly adjacent to each other so that the center-to-center distance is 21 μm as well, which is close to cellular resolution in the RGC layer. Figure 2 and movie 2 show examples of spontaneous waves recorded from the mouse retina at P3, P5 and P11 (with permission from A. Maccione, M. Gandolfo, M. Hennig, S. Eglen, L. Berdondini and E. Sernagor). With the APS MEA, retinal waves can now be investigated with unprecedented spatiotemporal resolution. This will lead to new insights about the mechanisms controlling developmental changes in the dynamics of the waves.



3. Cellular mechanisms underlying wave generation in the developing retina

Retinal waves are not a single phenomenon; they change fundamentally as the retina matures, so that even though immediate neighboring cells may keep firing in synchrony throughout development, the mechanisms responsible for such cooperation between neighbors completely change with development.

In mammals, retinal waves have been divided into three distinct pharmacological stages: Stage I (mediated by gap junctions), Stage II (mediated by acetylcholine) and Stage III (mediated by glutamate). In reptiles and birds, the distinction between stages is somewhat less conspicuous (see below).

3.1 Stage I waves - gap junctions

Gap junctional communication plays important roles during early development. Hence various types of connexins (Cx), the building blocks of gap junction channels, are expressed early in development, even before synapse formation. Cx 26, 32 and 43 are all expressed in the immature chick retina and their expression changes with development (reviewed in Becker, D. et al. 1998). As a general rule, these connexins are expressed at transient high levels during embryogenesis, while cells proliferate and migrate, and during the early period of spontaneous correlated activity. Subsequently, their expression falls to adult levels towards late gestational stages. In mouse, Cx 43 is already present at mid-gestation in the neural retina (Yancey, S. B. et al. 1992). Cx 36 and 43 increase with development, whilst at the same time, Cx 45 expression is downregulated (Kihara, A. H. et al. 2006). All three reach their mature expression two months post-natal.

Although gap junctions do not appear to play a significant role in generating correlated spontaneous activity in the immature inner retina after the onset of synaptogenesis (see next section), they seem to mediate the generation of propagating spontaneous calcium waves in the chick embryo retina several days prior to synapse formation

(Catsicas, M. et al. 1998). These early waves travel both horizontally across the RGC layer and vertically across the thickness of the retina, indicating that gap junctions are important for communication between retinal layers during early development. The role of these vertical waves remains to be unraveled.

Early gap junction-mediated waves have also been reported in mammals prior to synapse formation. In rabbit, lateral waves mediated by gap junction communication emerge at E22 and they last only one day (gestation lasts 31 days; Syed, M. M. et al. 2004a). These waves are large, frequent, about three times faster (about 450 $\mu\text{m/s}$) than later waves and they can overlap; they do not have a refractory period imposing spatial boundaries between subsequent waves as seen in Stage II waves (see below). Spontaneous lateral waves were also reported in the ventricular zone in rabbit (Syed, M. M. et al. 2004b). Although these waves were recorded at a time when synapses were already formed, they appear to be controlled by gap junctions as well as by cholinergic nicotinic and muscarinic connections.

In mouse, small clusters of cells displaying non propagating, correlated calcium increases independent of synaptic activation were observed at embryonic stages (between E16 and birth; gestation lasts 21 days in mouse) (Bansal, A. et al. 2000), suggesting that they may be mediated by gap junctional communication as well.

Both in chick and in rabbit, these early waves are impaired by drugs that affect gap junction coupling (gap junction blockers, dopamine, dopamine uptake inhibitors). Cyclic adenosine monophosphate (cAMP) also modulates these early waves. Indeed, they are blocked by adenosine receptor antagonists and their frequency increases in the presence of forskolin, an adenylate cyclase activator.

3.2 Stage II waves - cholinergic nicotinic connections

The first conventional synapses in the retina are reciprocal contacts between ACs in the IPL, and synapses from ACs to RGCs (see Robinson, S. R. 1991 for review). In mice, rats, rabbits and turtles, immunoreactivity for choline acetyltransferase (ChAT) can be detected already in the embryo (Hutchins, J. B. 1987; Nguyen, L. T. et al. 2000), and functional cholinergic connections appear around birth (Masland, R. H. 1977). These synapses originate from starburst amacrine cells (Masland, R. H. and Tsuchi, M. 1986; Vaney, D. I. 1990), and form a recurrently connected network that replaces the earlier gap junction network in initiating and propagating waves of activity. These initially depend solely on nicotinic cholinergic neurotransmission (Sernagor, E. and Grzywacz, N. M. 1996, 1999; Feller, M. B. et al. 1996; Catsicas, M. et al. 1998; Wong, R. O. L. et al. 1998; Bansal, A. et al. 2000; Sernagor, E. et al. 2000; Zhou, Z. J. and Zhao, D. 2000; Sernagor, E. et al. 2003; Syed, M. M. et al. 2004a), but are later also modulated by GABAergic and glutamatergic transmission (see below).

In all mammalian species investigated, stage II waves have a range of very salient properties, underpinning their importance as developmental cues during visual system development. First, their propagation speed of 150-250 $\mu\text{m/s}$ is much slower than stage I waves, and they occur less frequently, with inter-wave intervals of about one minute. Second, wave initiation points and trajectories are highly random, and over time, successive waves tile the entire retina with activity (Feller, M. B. et al. 1997). A study using high-density MEAs however also reported that stage II wave propagation is

biased in the nasal-temporal axis (Stafford, B. K. et al. 2009). Third, spatio-temporal wave properties such as wave size and duration are also highly random, ranging from very brief events only involving few neurons to long-lasting, large waves that cover large areas (Feller, M. B. et al. 1997; Hennig, M. H. et al. 2009). In fact, in the mouse and turtle retina it has been shown that early stage II wave sizes and durations assume power law distributions (Hennig, M. H. et al. 2009). On a given retinal area, small waves appear most frequently, and larger waves become progressively less frequent. This entails maximal variability in the spatio-temporal distribution of neural activity, and may be a prerequisite for the unbiased development of ordered RGC projections (Albert, M. V. et al. 2008).

Of all different developmental stages, the mechanisms underlying cholinergic waves are currently best understood. Even though it was known for a long time that nicotinic transmission between starburst ACs supports their propagation, it has proved difficult to understand how exactly their spatio-temporal properties arise. In particular, neural activity is highly correlated during waves, suggesting strong recurrent synapses that rapidly synchronize large neuronal populations. At the same time, activity is also strongly desynchronized on longer time scales, as reflected by the high degree of randomness of wave initiation points, trajectories, sizes, durations and inter-wave intervals. Computational models of neuronal networks have shown that this co-existence of synchrony and randomness is not expected in systems of simple coupled oscillators (e.g. Hansel, D. et al. 1995), indicating that the specific physiological properties of starburst ACs are of particular importance.

Experimental and theoretical work has therefore focused on the questions of how waves are initiated and which mechanisms regulate their propagation. A first key insight came from a computational model by Feller, M. B. et al. (1997). In this study, it was suggested that waves are initiated by cell-autonomous, spontaneous activity in ACs, which then propagate laterally through reciprocal synaptic connections between ACs. This activity is then read out and transmitted on by RGCs. Importantly, depolarizations were hypothesized to drive ACs into a long-lasting refractory state, preventing wave propagation into previously active regions. This model could therefore explain why subsequent waves usually do not overlap and, over time, tile the retina with activity. Only non-refractory regions can be invaded by an active wave, such that recent history of activity limits the spreading of subsequent waves. Therefore, the fraction of non-refractory, recruitable ACs was identified as the central parameter that determines wave properties (Butts, D. A. et al. 1999).

These hypothesized mechanisms were later confirmed with patch clamp recordings from starburst ACs in the rabbit retina by Zheng, J. J. et al. (2006). These experiments showed that starburst ACs produce cell-autonomous spontaneous calcium bursts even when all neurotransmission is blocked. A propagating wave is triggered when multiple, coincident spontaneous bursts in nearby neurons occur. This explanation is also supported by a more recent modeling study (Hennig, M. H. et al. 2009). Moreover, cell-autonomous bursts, as well as stronger and more prolonged calcium bursts during waves, are followed by a strong slow afterhyperpolarization (sAHP), which constitutes a potent refractory mechanism lasting tens of seconds (Zheng, J. J. et al. 2006). Pharmacological investigation revealed that the sAHP is mediated by calcium-dependent potassium conductance that is, similar to the sAHP conductance identified in cortical neurons (e.g. Abel, H. J. et al. 2004), suppressed by cAMP and the adenylate cyclase activator forskolin.

The recordings by Zheng, J. J. et al. (2006) also demonstrated that strong calcium bursts during waves lead to a stronger and more sustained sAHP than weaker cell-autonomous bursts. Two modeling studies showed subsequently that this dependency of the refractory mechanism on the recent history of activity is critical for creating the high variability and randomness of stage II waves (Godfrey, K. B. and Swindale, N. V. 2007; Hennig, M. H. et al. 2009). In particular, this variability arises from competition between synchronization due to strong synaptic contacts and the desynchronizing effect of the refractory mechanism. Random cell-autonomous bursts, which cause a short refractory period, and waves, which lead to longer-lasting refractoriness, constantly disrupt the effective network connectivity. As a result, the number of non-refractory neurons fluctuates strongly, as does the area available to propagating activity (Hennig, M. H. et al. 2009). Depending on the strength of the refractory mechanism or the frequency ratio of cell-autonomous bursts versus waves, the network is either in a synchronized state characterized by prevalence of large waves, or in a desynchronized state where only small waves are observed. A comparison of data from mice and turtles indicated that stage II waves are generated very close to the transition point between these two states, where the network supports waves of all sizes, and their size distribution closely approximates a power law.

Interestingly, experiments have shown that modulation of the sAHP can shift the network into either one of these two regimes. An increase of cAMP levels either with forskolin or by activating adenosine A2 receptors, which both reduce the strength the sAHP (Zheng, J. J. et al. 2006), increases wave size and frequency (Stellwagen, D. et al. 1999). Inhibiting adenylate cyclase or PKA activity, on the other hand, which enhances the effect of the sAHP, has the opposite effect, leading to smaller, less frequent waves (Stellwagen, D. et al. 1999). Both effects could be directly reproduced by the model of Hennig, M. H. et al. (2009), supporting the idea that maximum variability is indeed a key property of Stage II.

3.3 Stage III waves - glutamatergic connections

At later stages, mammalian wave control switches from acetylcholine to glutamate (Bansal, A. et al. 2000; Zhou, Z. J. and Zhao, D. 2000; Syed, M. M. et al. 2004a; Blankenship, A. G. et al. 2009). The transition from cholinergic to glutamatergic control takes place at the time when bipolar cell axon terminals establish connections in the IPL. In mouse this occurs around P10 (Fisher, L. J. 1979; Morgan, J. L. et al. 2008), shortly before eye opening (P12), when the first light driven responses are detected in RGCs (Demas, J. et al. 2003; Tian, N. and Copenhagen, D. R. 2001). In rabbit, the transition occurs between P3-4 (Syed, M. M. et al. 2004a). At the same time, reciprocal cholinergic connections between starburst ACs withdraw (Zheng, J. J. et al. 2004), bringing an end to Stage II cholinergic waves. Waves completely disappear (between P14-21 in mouse, beyond P7 in rabbit), once vision becomes functional.

Waves also switch from cholinergic to glutamatergic control in chick, but the transition is less abrupt, with a short period during which both neurotransmitters overlap in controlling the waves (from E14-15; Wong, R. O. L. et al. 1998; Sernagor, E. et al. 2000). In turtle, on the other hand, both neurotransmitters are required virtually at all times. When waves emerge at embryonic stage (S) 22, three weeks before hatching, they are mediated by cholinergic nicotinic connections, but glutamate becomes involved about three days later, at S23 (Sernagor, E. and Grzywacz, N. M. 1999; Sernagor, E. et al. 2003), when RGCs

become driven by light (Sernagor, E. and Grzywacz, N. M. 1995). These differences probably reflect how much these different species rely on vision at birth. Rodents are born “blind” and entirely rely on maternal protection until eye opening, at P12. Interestingly, waves emerge much earlier in macaques (Warland, D. K. et al. 2006): they are already present at E60 and start to wane by E76, long before birth (gestation lasts about 165 days). Both reptiles and birds have a fully functional retina at birth, and as a matter of fact they rely mostly on vision to orient themselves and escape predators as soon as they hatch.

In turtle, ferret and rabbit, the contribution of glutamate is largely mediated by AMPA/kainate rather than by NMDA receptors (Sernagor, E. and Grzywacz, N. M. 1999; Wong, R. O. L. et al. 2000; Sernagor, E. et al. 2003; Syed, M. M. et al. 2004a), while in chick and mouse it appears to be mediated equally by both subtypes (Wong, R. O. L. et al. 1998; Sernagor, E. et al. 2000; Blankenship, A. G. et al. 2009).

The source of glutamate responsible for the generation of Stage III waves is not known. The possible candidates are bipolar and photoreceptor terminals, RGC axon collaterals or even amacrine cells. In the rodent retina, VGLUT1, the vesicular transporter found in bipolar and photoreceptor terminals appears before the formation of mature ribbon synapses and the emergence of light responses (rat: P5-7 in the OPL and P7 in the IPL; mouse: P3 in the OPL and P5 in the IPL), reaching its adult pattern by P14. VGLUT1 is thus already functional when spontaneous glutamatergic postsynaptic responses begin (Tian, N. and Copenhagen, D. R. 2001), demonstrating the existence of vesicular glutamatergic release in the IPL before the maturation of ribbon synapses. One possible explanation for the presence of this early glutamatergic neurotransmission is that it originates in photoreceptors, known to make transient connections in the IPL at early stages, during the period of spontaneous activity (Johnson, J. et al. 1999). The central role played by VGLUT1 in wave generation was demonstrated by showing that waves occurring after P10 cannot be blocked by AMPA/kainate or NMDA antagonists in mice lacking VGLUT1 (Blankenship, A. G. et al. 2009). Interestingly, these waves are completely abolished by cholinergic nicotinic blockers, suggesting that Stage II waves can persist when the mechanism mediating Stage III waves is impaired (see section on homeostatic plasticity). The fact that Stage II waves persist and replace glutamate-mediated waves in VGLUT1 mutants demonstrates that release from BCs or from photoreceptor terminals mediates Stage III waves. (The authors of that study argue against the possibility that release is from photoreceptor terminals because their experiments were performed in bright light, when photoreceptors are hyperpolarized, hence releasing less glutamate). The same study also showed that a significant amount of glutamate release during the waves originates from extrasynaptic spillover. Enhancing glutamate spillover by blocking glutamate transporters increases the frequency of occurrence of the waves.

Interestingly, RGC bodies already start expressing VGLUT2 at very early stages (P0 in rat) (Stella, S. L. Jr. et al. 2008), suggesting that paracrine glutamatergic release may occur in the RGC layer. VGLUT2 expression is also found in the IPL from P10, corresponding with the onset of Stage III waves. Hence, glutamate release from RGC axon collaterals may also contribute to Stage III waves. Such collaterals have been observed in the immature retina (Sernagor, E. et al. 2001).

In the mature retina, there is a class of ACs which express VGLUT3 (Haverkamp, S. and Wässle, H. 2004; Johnson, P. T. et al. 2004) and make connections with RGCs. The mere fact that there are no glutamatergic waves in VGLUT1^{-/-} mice (Blankenship, A. G. et al. 2009) already suggests that these ACs are unlikely to provide the source of glutamate generating Stage III waves. Moreover, VGLUT3 colocalizes with the

metabotropic glutamate receptor 4 (mGluR4) (Johnson, J. et al. 2004), but not with ionotropic glutamatergic receptors, hence it is unlikely to be linked to Stage III waves.

Intriguingly, despite the fact that the networks generating Stage II and Stage III waves in mammals are so different, at present there is no convincing evidence that the spatiotemporal properties of these waves change significantly. In rabbit, Stage III waves were reported to appear at lower frequency, to be either more restricted spatially or completely stationary (Syed, M. M. et al. 2004a). In mouse on the other hand, Stage III waves were reported to have the same frequency as Stage II waves but they occurred in groups of repetitive waves, each time in a different retinal area (Blankenship, A. G. et al. 2009). These waves propagated with great speed variability but the speeds were not fundamentally different from those observed at Stage II. Both studies were performed with calcium imaging using low frame acquisition rate (0.9-4 frames/s). On the other hand, when recorded with a 60 electrodes MEA, the speeds of Stage III mouse waves were significantly faster (Demas, J. et al. 2003). Speeds were also faster in chick and turtle studies using calcium imaging performed at 25-30 frames/s. These discrepancies reinforce the fact that extreme caution should be taken when trying to quantify fine grained spatiotemporal parameters. Fast/short events are bound to be either missed or miscalculated when acquisition is performed at low spatiotemporal resolution.

Cross correlation calculations derived from multi-unit recordings in turtle with a single extracellular electrode suggest that acetylcholine mediates lateral propagation while glutamate may be involved in local excitability but does not regulate propagation *per se* (Sernagor, E. and Grzywacz, N. M. 1999). Calcium imaging studies (performed at 25-30 Hz) in chick and turtle confirmed that acetylcholine influences the wave spatial extent, whereas glutamate modulates their speed (Sernagor, E. et al. 2000; Sernagor, E. et al. 2003). This implies that Stage III waves should be faster and spatially more restricted in mammals, reflecting the recruitment of bipolar terminals (the axon terminals of these cells have a narrower span than amacrine terminals). However, this remains to be confirmed experimentally using a more rigorous approach to decipher the spatiotemporal features of the activity at different stages of development.

3.4 The role of synaptic inhibition in retinal waves

So far we have considered only the excitatory component of the waves. However, synaptic inhibition is also implicated in wave modulation in an age-dependent manner, with stronger involvement at late stages of development, when spontaneous correlated activity begins to disappear.

The developmental expression of VGAT, the vesicular transporter for GABA and glycine, precedes that of VGLUT1 by several days in the postnatal rodent retina (Johnson, J. et al. 2003). Indeed, VGAT is already present in the IPL by P1, suggesting that vesicular GABAergic and/or glycinergic neurotransmission at conventional synapses occurs in the IPL before glutamatergic synapses become functional. In support, several studies have demonstrated an important role for GABAergic neurotransmission in controlling the generation and propagation of retinal waves.

Like in many other parts of the immature CNS (Ben-Ari, Y. et al. 2007; Wang, D. D. and Kriegstein, A. R. 2009), activation of GABA type-A receptors exerts a depolarizing effect on immature RGCs and ACs (Sernagor, E. et al. 2003; Zhang, L. L. et al. 2006; Wang, C. T. et al. 2007). Activation of synaptic and/or extrasynaptic GABA_A receptors causes shunting inhibition in these cells, clamping their membrane potential around -40mV, the

reversal potential for chloride in immature neurons (Wang, C. T. et al. 2007). However, GABAergic neurotransmission does not affect the wave spatiotemporal properties at early stages (Stellwagen, D. et al. 1999; Sernagor, E. et al. 2003). In turtle, waves emerge at S22, three weeks before hatching, and they are not affected by the GABA_A antagonist bicuculline until S25, one week prior to hatching (Sernagor, E. et al. 2003). In mouse, GABAergic ACs become involved in wave modulation around P4-5, once they show clear stratification in the inner plexiform layer (Zhang, L. L. et al. 2006). At that time, GABA_A responses are depolarizing in RGCs and ACs somata, abruptly shifting to mature hyperpolarization between P6-P7. GABA and glycine, which also participate in wave modulation at later stages, shift from being functionally excitatory to inhibitory (GABA – ferret: Fischer, K. F. et al. 1998; turtle: Sernagor, E. et al. 2003; rabbit: Zheng, J. J. et al. 2004; glycine – rabbit: Zhou, Z. J. 2001).

Once GABA_A signaling becomes involved in the circuitry controlling the spatiotemporal features of the waves, it has a strong influence on the dynamics of the activity. In a longitudinal study of the changes in retinal wave dynamics in turtle spanning the last three gestational weeks (gestation takes eight weeks) until a month post-hatching, waves dramatically slow down and become narrower once GABA_A signaling becomes involved at S25, a week before hatching. Towards hatching they become patches within which RGCs fire in near synchrony, occurring at random both in time and in location (Sernagor, E. et al. 2003; see also Sernagor, E. et al. 2001, Sernagor, E. and Mehta, V. 2001). These patches become smaller and eventually disappear about one month post-hatching. This gradual restriction in lateral propagation is due to developmental changes in the polarity of GABA_A responses (Sernagor, E. et al. 2003). When GABA synthesis is impaired (by blocking glutamic acid decarboxylase, the enzyme that synthesizes GABA) at post-hatching stages, leading to a decrease in endogenous GABA, spontaneous activity is stronger than in age-matched controls, and it even exhibits propagation (Sernagor, E. 2006), presumably because the synaptic network generating spontaneous activity is relieved from GABAergic inhibition. Interestingly, depleting the retina from endogenous GABA has the opposite effect at S25, when GABA is excitatory: waves completely disappear, suggesting that endogenous depolarizing GABA provides tonic excitation that is necessary to generate the waves. These developmental changes in the dynamics of spontaneous activity coincide with the upregulation in the IPL of the potassium-chloride membrane cotransporter KCC2 that extrudes chloride from mature cells (Sernagor, E. et al. 2003), thereby causing the equilibrium potential for chloride to shift to more hyperpolarized levels, so that GABA_A responses become inhibitory.

Several studies suggest that GABA is important in controlling the disappearance of retinal waves in mammals as well. An elegant study employing dual patch-clamp recordings and calcium imaging from pairs of rabbit starburst ACs has shown that these cells make reciprocal GABAergic synapses with each other, and that the GABAergic responses switch from excitation to inhibition while the waves disappear, (Zheng, J. J. et al. 2004; see also Zhou, Z. J. 2001; Syed, M. M. et al. 2004a). Moreover, GABA shifts from excitation to inhibition around P15-18 in ferret (Fischer, K. F. et al. 1998), shortly before waves stop propagating (Wong, R. O. L. et al. 1993). Finally, KCC2 expression increases significantly during the second week of postnatal development in rat (Vu, T. Q. et al. 2000) and mouse (Zhang, L. L. et al. 2006, 2007). Interestingly, in the retina the developmental hyperpolarizing shift in chloride-mediated GABA_A responses is not mediated by downregulation of NKCC1, a transporter known to accumulate chloride in early development in other parts of the nervous system (Zhang, L. L. et al. 2007). Apart from changes in the spatial patterns of activity, GABA-mediated developmental

changes in the temporal firing patterns have been reported in ferret RGCs (Wong, R. O. L. and Oakley, D. M. 1996). Once eye-specific segregation is complete in the LGN, On and Off RGCs develop distinct firing patterns, although both cell types still burst in synchrony. On cells adopt a much lower burst frequency compared to Off cells. The emergence of these different On-Off rhythms occurs when GABA becomes inhibitory and suppresses bursting activity in On RGCs more effectively than in Off RGCs (Fischer, K. F. et al. 1998).

In all species, waves disappear shortly after the onset of visual experience, when RGCs become driven by light, suggesting that exposure to light at birth somehow triggers a mechanism that leads to the disappearance of the waves. In the absence of visual experience, spontaneous bursting activity in turtle RGCs (Sernagor, E. and Grzywacz, N. M. 1996) and waves (Sernagor, E. et al. 2003) persist for longer periods post-hatching, suggesting that early visual experience may indeed trigger their disappearance. At the same time, GABA does not switch polarity, KCC2 expression is lower and waves keep propagating following one month of rearing in the dark (Sernagor, E. et al. 2003), suggesting that light may have a direct effect on the shift in polarity of GABA_A responses. However, dark-rearing does not prevent the disappearance of retinal waves in mouse (Demas, J. et al. 2003) even though it reversibly suppresses the post-eye opening surge in spontaneous synaptic events (Tian, N. and Copenhagen, D. R. 2001). Hence, the effects of dark-rearing upon correlated activity vary from species to species, presumably depending on how much they rely on visual experience during early postnatal life.

Since GABA has such a strong impact on age-related changes in spontaneous activity patterns, it is important to understand what factors (other than exposure to light) control developmental changes in GABA_A activity. Sustained GABA_A activity is required for GABA to shift polarity in rodent hippocampal cultures (Ganguly, K. et al. 2001). In the turtle retina, chronic blockade of GABA_A receptors *in vivo* during the period of the switch prevents GABA_A responses from shifting polarity and KCC2 expression remains lower, resulting in the persistence of strong spontaneous waves at one month post-hatching, when normally there is no correlated activity anymore (Leitch, E. et al. 2005).

Although GABA_B signaling does not influence waves late in development, endogenous GABA_B activity has been shown to exert a strong inhibitory effect on Stage I waves in rabbit (Syed, M. M. et al. 2004a) and in pre-synaptogenesis waves in chick (Catsicas, M. and Mobbs, P. 2001). The effect is through downregulation of calcium channels rather than directly on potassium channels (Catsicas, M. and Mobbs, P. 2001) and it completely disappears once waves switch to Stage II. The role of this early inhibitory GABA_B effect on retinal maturation remains to be deciphered.

Finally, glycinergic signaling has also been demonstrated to modulate late waves in chick. Blockade of glycinergic receptors with strychnine reduces the wave frequency at E16 (Wong, R. O. L. et al. 1998), and intriguingly it has the same effect on early waves before synapse formation (Catsicas, M. et al. 1998), suggesting that glycine has an excitatory effect on wave generation throughout a long developmental period.

4. Retinal waves are under homeostatic control

In this chapter, we have seen that retinal waves are virtually universal in the developing vertebrate retina, at least in reptiles, birds and mammals. We have also seen that despite some interspecies differences, by and large these waves are relatively similar across species (Hennig, M. H. et al. 2009). They are initially mediated via gap junctional communication, followed by cholinergic signaling and later by glutamatergic synapses. The maturation of synaptic inhibition is involved in modulation of the wave dynamics at later developmental stages, and may even be responsible for their disappearance. These remarkable similarities hint to the fact that retinal waves are a very robust

phenomenon, resilient to changes imposed by the environment (Blankenship, A. G. and Feller, M. B. 2010). Indeed, under both acute pharmacological conditions and genetic manipulations which block the waves, propagating activity can resume, albeit controlled by alternative cellular mechanisms.

In rabbit, waves switch from Stage I to Stage II between E22 and E23. Immediately after the switch, Stage I waves can resume following acute blockade of Stage II waves with a cholinergic nicotinic antagonist (Syed, M. M. et al. 2004a), demonstrating the capacity for homeostatic plasticity during the transition period. However, this ability to reinstate waves is short lived and was not observed at later developmental stages. Moreover, it is not known whether prolonged blockade of Stage I waves with gap junction blockers would trigger the premature appearance of Stage II waves.

In an attempt to investigate the role of cholinergic waves in guiding the wiring of connections in the visual system, many studies have used a transgenic mouse in which the subunit $\beta 2$ of the cholinergic nicotinic receptor has been deleted (Bansal, A. et al. 2000). Although it was initially thought that there are no waves during the first postnatal week in the $\beta 2^{-/-}$ mutant (Bansal, A. et al. 2000; Muir-Robinson, G. et al. 2002; McLaughlin, T. et al. 2003), more recent studies have shown that under appropriate experimental conditions (the temperature of the experimental chamber has to be above 35°C), waves actually never disappear in that mouse (Sun, C. et al. 2008; Stafford, B. K. et al. 2009). However, as in the case of acute blockade of Stage II waves in rabbit described above, these waves (recorded at P5-6) actually are Stage I-like, as indicated by their spatiotemporal properties and pharmacological sensitivity to gap junction blockers. They occur at higher frequency, they are larger and faster than in wild type (Stafford, B. K. et al. 2009). Importantly, Stage III glutamatergic waves emerge earlier than normal in the $\beta 2^{-/-}$ mouse (at P8) (Bansal, A. et al. 2000). These results demonstrate that the three consecutive mechanisms mediating retinal waves are intimately linked and can compensate for each other.

Similar results were obtained when using a conditional mutant in which choline acetyltransferase (ChAT), the enzyme that synthesizes acetylcholine was deleted from large retinal areas (Stacy, R. C. et al. 2005). No waves could be detected in these areas for the first few postnatal days, but they resumed by P5. These waves were mediated by gap junctions, but not by glutamate. Here, interestingly, in contrast to rabbit (Syed, M. M. et al. 2004a), small patches of synchronized activity could be detected in P5 wild type retinas following 5-10 hours of continuous pharmacological blockade of cholinergic nicotinic receptors.

Finally, abolishing Stage III glutamatergic waves in VGLUT1^{-/-} mice induces the reappearance of Stage II-like cholinergic waves (Wang, C. T. et al. 2007).

In conclusion, the findings presented in this section demonstrate the existence of powerful homeostatic compensation mechanisms in the developing retina. These mechanisms ensure that the retina remains spontaneously active throughout the period during which it is supposed to exhibit waves. Blockade of one form of activity induces the reappearance of the previous mechanism (e.g. blockade of Stage II waves induces the re-emergence of Stage I waves and blockade of Stage III waves induces the re-emergence of Stage II waves). At the same time, blockade of Stage II waves (at least through genetic deletion) can also induce the premature development of glutamatergic connections mediating Stage III waves.

References

- Abel, H. J., Lee, J. C., Callaway, J. C. and Foehring, R. C. 2004. Relationships between intracellular calcium and afterhyperpolarizations in neocortical pyramidal neurons. *J. Neurophysiol.* 91, 324–335.
- Albert, M. V., Schnabel, A. and Field, D. J. 2008. Innate visual learning through spontaneous activity patterns. *PLoS Comput. Biol.* 4(8), e1000137.
- Bansal, A., Singer, J. H., Hwang, B. J., Xu, W., Beaudet, A. and Feller, M. B. 2000. Mice lacking specific nicotinic acetylcholine receptor subunits exhibit dramatically altered spontaneous activity patterns and reveal a limited role for retinal waves in forming on and off circuits in the inner retina. *J. Neurosci.* 20, 7672-7681.
- Becker, D., Bonness, V. and Mobbs, P. 1998. Cell coupling in the retina: patterns and purpose. *Cell. Biol. Internat.* 22, 781-792.
- Berdondini, L., Imfeld, K., Maccione, A., Tedesco, M., Neukom S., Koudelka-Hep, M. and Martinoia, S. 2009. Active pixel sensor array for high spatio-temporal resolution electrophysiological recordings from single cell to large scale neuronal networks. *Lab Chip* 9, 2644-2651.
- Ben-Ari, Y., Gaiarsa, J. L., Tyzio, R. and Khazipov, R. 2007. GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol. Rev.* 87, 1215-1284.
- Blankenship, A. G., Ford, K. J., Johnson, J., Seal, R. P., Edwards, R. H., Copenhagen, D. R. and Feller, M. B. 2009. Synaptic and extrasynaptic factors governing glutamatergic retinal waves. *Neuron* 62, 230-241.
- Blankenship, A. G. and Feller, M. B. 2010. Mechanisms underlying spontaneous patterned activity in developing neural circuits. *Nat. Rev. Neurosci.* 11, 18-29.
- Butts, D. A., Feller, M. B., Shatz, C. J. and Rokhsar, D. S. 1999. Retinal waves are governed by collective network properties. *J. Neurosci.* 19, 3580–3593.
- Catsicas, M., Bonness, V., Becker, D. L. and Mobbs, P. 1998. Spontaneous Ca²⁺ waves and their transmission in the developing chick retina. *Curr. Biol.* 8, 283-286.
- Catsicas, M. and Mobbs, P. 2001. GABA_B receptors regulate chick retinal calcium waves. *J. Neurosci.* 21, 897-910.
- Demas, J., Eglén, S. J. and Wong, R. O. L. 2003. Developmental loss of synchronous spontaneous activity in the mouse retina is independent of visual experience. *J. Neurosci.* 23, 2851-2860.
- Díez-García, J., Akemann, W. and Knöpfel T. 2007. *In vivo* calcium imaging from genetically specified target cells in mouse cerebellum. *Neuroimage* 34, 859-869.

Feller, M. B. 1999. Spontaneous correlated activity in developing neural circuits. *Neuron* 22, 653-656.

Feller, M. B., Wellis, D. P., Stellwagen, D., Werblin, F. S. and Shatz, C. J. 1996. Requirement for cholinergic synaptic transmission in the propagation of spontaneous retinal waves. *Science* 272, 1182-1187.

Feller, M. B., Butts, D. A., Aaron, H. L., Rokhsar, D. S. and Shatz, C. J. 1997. Dynamic processes shape spatiotemporal properties of retinal waves. *Neuron* 19, 293-306.

Fisher, L. J. 1979. Development of synaptic arrays in the inner plexiform layer of neonatal mouse retina. *J. Comp. Neurol.* 187, 359-372.

Fischer, K. F., Lukasiewicz, P. D. and Wong, R. O. L. 1998. Age-dependent and cell class-specific modulation of retinal ganglion cell bursting activity by GABA. *J. Neurosci.* 18, 3767-3778.

Galli, L. and Maffei, L. (1988) Spontaneous impulse activity of rat retinal ganglion cells in prenatal life. *Science* 24, 90-91.

Ganguly, K., Schinder, A. F., Wong, S. T. and Poo, M. 2001. GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. *Cell* 18, 521-532.

Godfrey, K. B. and Swindale, N. V. 2007. Retinal wave behavior through activity-dependent refractory periods. *PLoS Comput. Biol.* 3, e245.

Grzywacz, N. M. and Sernagor, E. 2000 Spontaneous activity in developing turtle retinal ganglion cells: statistical analysis. *Vis. Neurosci.* 17, 229-241.

Hansel, D., Mato, G. and Meunier, C. 1995. Synchrony in excitatory neural networks. *Neural computation* 7, 307-737.

Haverkamp, S. and Wässle, H. 2004. Characterization of an amacrine cell type of the mammalian retina immunoreactive for vesicular glutamate transporter 3. *J. Comp. Neurol.* 468, 251-263.

Hennig, M. H., Adams, C., Willshaw, D. and Sernagor, E. 2009. Early-stage waves in the retinal network emerge close to a critical state transition between local and global functional connectivity. *J. Neurosci.* 29, 1077-1086.

Hutchins, J. B. 1987 Review: Acetylcholine as a neurotransmitter in the vertebrate retina. *Exp. Brain Res.* 45, 1-38.

Johnson, J., Tian, N., Caywood, M. S., Reimer, R. J., Edwards, R. H. and Copenhagen, D. R. 2003. Vesicular neurotransmitter transporter expression in developing postnatal rodent retina: GABA and glycine precede glutamate. *J. Neurosci.* 23, 518-529.

Johnson, J., Sherry, D. M., Liu, X., Fremeau, R. T. Jr., Seal, R. P., Edwards, R. H. and Copenhagen, D. R. 2004. Vesicular glutamate transporter 3 expression identifies glutamatergic amacrine cells in the rodent retina. *J. Comp. Neurol.* 477, 386-398.

Johnson, P. T., Williams, R. R., Cusato, K. and Reese, B. E. 1999. Rods and cones project to the inner plexiform layer during development. *J. Comp. Neurol.* 414, 1-12.

Katz, L. C. and Shatz, C. J. 1996 Synaptic activity and the construction of cortical circuits. *Science* 274, 1133-1138.

Kihara, A. H., Mantovani de Castro, L., Belmonte, M. A., Yan, C. Y., Moriscot, A. S. and Hamassaki, D. E. 2006. Expression of connexins 36, 43, and 45 during postnatal development of the mouse retina. *J. Neurobiol.* 66, 1397-410.

Leitch, E., Coaker, J., Young, C., Mehta, V. and Sernagor, E. 2005. GABA type-A activity controls its own developmental polarity switch in the maturing retina. *J. Neurosci.* 25, 4801-4805.

Maffei, L. and Galli-Resta, L. 1990. Correlation in the discharges of neighboring rat retinal ganglion cells during prenatal life. *Proc. Natl. Acad. Sci. U.S.A.* 87, 2861-2864.

Masland, R. H. 1977. Maturation of function in the developing rabbit retina. *J. Comp. Neurol.* 175, 275-286.

Masland, R. H. and Tauchi, M. 1986. The cholinergic amacrine cells. *Trends Neurosci.* 9, 218-223.

McLaughlin, T., Torborg, C. L., Feller, M. B. and O'Leary, D. M. 2003. Retinotopic map refinement requires spontaneous retinal waves during a brief critical period of development. *Neuron* 40, 1147-1160.

Meister, M., Wong, R. O. L., Baylor, D. A. and Shatz, C. J. 1991. Synchronous bursts of action potentials in ganglion cells of the developing mammalian retina. *Science* 252, 939-943.

Morgan, J. L., Schubert, T. and Wong, R. O. 2008. Developmental patterning of glutamatergic synapses onto retinal ganglion cells. *Neural Dev.* 3, 8.

Muir-Robinson, G., Hwang, B. J. and Feller, M. B. 2002. Retinogeniculate axons undergo eye-specific segregation in the absence of eye-specific layers. *J. Neurosci.* 22, 5259-5264.

Nguyen, L. T., De Juan, J., Mejia, M. and Grzywacz, N. M. 2000. Localization of choline acetyltransferase in the developing and adult turtle retinas. *J. Comp. Neurol.* 420, 512-526.

O'Donovan, M. J. 1999. The origin of spontaneous activity in developing networks of the vertebrate nervous system. *Curr. Opin. Neurobiol.* 9, 94-104.

Robinson, S. R. 1991. Development of the mammalian retina. In: *Neuroanatomy of the visual pathways and their development* (ed. B. Dreher and S.R. Robinson), Macmillan.

Sernagor, E. 2006. Synaptogenesis and early neural activity. In: *Retinal Development* (ed. E. Sernagor, S.J. Eglén, W. Harris and R.O. Wong), p. 265, Cambridge University Press

Sernagor, E and Grzywacz, N. M. 1995. Emergence of complex receptive field properties of ganglion cells in the developing turtle retina. *J. Neurophysiol.* 73, 1355-1364.

Sernagor, E. and Grzywacz, N. M. 1996. Influence of spontaneous activity and visual experience on developing retinal receptive-fields. *Curr. Biol.* 6, 1503-1508.

Sernagor, E. and Grzywacz, N. M. 1999. Spontaneous activity in developing turtle retinal ganglion cells: pharmacological studies. *J. Neurosci.* 19, 3874-3887.

Sernagor, E., Eglén, S. J. and O'Donovan, M. J. 2000. Differential effects of acetylcholine and glutamate blockade on the spatiotemporal dynamics of retinal waves. *J. Neurosci.* 20(RC56), 1-6.

Sernagor, E., Eglén, S. J. and Wong, R. O. L. 2001. Development of retinal ganglion cell structure and function. *Prog. Retin. Eye Res.* 20, 139-174.

Sernagor, E. and Mehta, V. 2001. The role of early neural activity in the maturation of turtle retinal function. *J. Anat.* 199: 375-383.

Sernagor, E., Young, C. and Eglén, S. J. 2003. Developmental modulation of retinal wave dynamics: shedding light on the GABA saga. *J. Neurosci.* 23, 7621-7629.

Stacy, R. C., Demas, J., Burgess, R. W., Sanes, J. R. and Wong, R. O. L. 2005. Disruption and recovery of patterned retinal activity in the absence of acetylcholine. *J. Neurosci.* 25, 9347-9357.

Stafford, B. K., Sher, A., Litke, A. M. and Feldheim, D. A. 2009. Spatial-temporal patterns of retinal waves underlying activity-dependent refinement of retinofugal projections. *Neuron* 64, 200-212.

Stella, S. L. Jr., Li, S., Sabatini, A., Vila, A. and Brecha, N. C. 2008. Comparison of the ontogeny of the vesicular glutamate transporter 3 (VGLUT3) with VGLUT1 and VGLUT2 in the rat retina. *Brain Res.* 1215, 20-29.

Stellwagen, D., Shatz, C. J. and Feller, M. B. 1999. Dynamics of retinal waves are controlled by cyclic AMP. *Neuron* 24, 673-685.

Sun, C., Warland, D. K., Ballesteros, J. M., van der List, D. and Chalupa, L. M. 2008. Retinal waves in mice lacking the beta2 subunit of the nicotinic acetylcholine receptor. *Proc. Natl. Acad. Sci. U.S.A.* 105, 13638-13643.

Syed, M. M., Lee, S., Zheng, J. and Zhou, Z. J. 2004a. Stage-dependent dynamics and modulation of spontaneous waves in the developing rabbit retina. *J. Physiol.* 560, 533-549.

Syed, M. M., Lee, S., He, S. and Zhou, Z. J. 2004b. Spontaneous waves in the ventricular zone of developing mammalian retina. *J. Neurophysiol.* 91, 1999-2009.

Tian, N. and Copenhagen, D. R. 2001. Visual deprivation alters development of synaptic function in inner retina after eye opening. *Neuron* 32, 439-449.

Tootle, J. S. 1993. Early postnatal development of visual function in ganglion cells of the

cat retina. *J. Neurophysiol.* 69, 1645-1660.

Torborg, C. L. and Feller, M. B. 2005. Spontaneous patterned retinal activity and the refinement of retinal projections. *Prog. Neurobiol.* 76, 213-235.

Vaney, D. I. 1990. The mosaic of amacrine cells in the mammalian retina. *Prog. Ret. Res.* 9, 49-100.

Vu. T. Q., Payne, J. A. and Copenhagen, D. R. 2000. Localization and developmental expression patterns of the neuronal K-Cl cotransporter (KCC2) in the rat retina. *J. Neurosci.* 20, 1414-1423.

Wang, C. T., Blankenship, A. G., Anishchenko, A., Elstrott, J., Fikhman, M., Nakanishi, S. and Feller, M. B. 2007. GABA(A) receptor-mediated signaling alters the structure of spontaneous activity in the developing retina. *J. Neurosci.* 27, 9130-9140.

Wang, D. D. and Kriegstein, A. R. 2009. Defining the role of GABA in cortical development. *J. Physiol.* 587, 1873-1879.

Warland, D. K., Huberman, A. D. and Chalupa, L. M. 2006. Dynamics of spontaneous activity in the fetal macaque retina during development of retinogeniculate pathways. *J. Neurosci.* 26, 5190-5197.

Wong, R. O. L. 1999. Retinal waves and visual system development. *Ann. Rev. Neurosci.* 22, 29-47.

Wong, R. O. L., Meister, M. and Shatz, C. J. 1993. Transient period of correlated bursting activity during development of the mammalian retina. *Neuron* 11, 923-938.

Wong, R. O. L., Chernjavsky, A., Smith, S. J. and Shatz, C. J. 1995. Early functional neural networks in the developing retina. *Nature* 374, 716-718.

Wong, R. O. L. and Oakley, D. M. 1996. Changing patterns of spontaneous bursting activity of on and off retinal ganglion cells. *Neuron* 16, 1087-1095.

Wong, W. T., Sanes, J. R. and Wong, R. O. L. 1998. Developmentally regulated spontaneous activity in the embryonic chick retina. *J. Neurosci.* 18, 8839-8852.

Yancey, S. B., Biswal, S. and Revel, J. P. 1992. Spatial and temporal patterns of distribution of the gap junction protein connexin43 during mouse gastrulation and organogenesis. *Development* 114, 203-212.

Zhang, L. L., Pathak, H. R., Coulter, D. A., Freed, M. A. and Vardi, N. 2006. Shift of intracellular chloride concentration in ganglion and amacrine cells of developing mouse retina. *J. Neurophysiol.* 95, 2404-2416.

Zhang, L. L., Delpire, E. and Vardi, N. 2007. NKCC1 does not accumulate chloride in developing retinal neurons. *J. Neurophysiol.* 98, 266-277.

Zheng, J. J., Lee, S. and Zhou, Z. J. 2004. A developmental switch in the excitability and function of the starburst network in the mammalian retina. *Neuron* 44, 851-864.

Zheng, J. J., Lee, S. and Zhou, Z. J. 2006. A transient network of intrinsically bursting starburst cells underlies the generation of retinal waves. *Nat. Neurosci.* 9, 363–371.

Zhou, Z. J. 2001. A critical role of the strychnine-sensitive glycinergic system in spontaneous retinal waves of the developing rabbit. *J. Neurosci.* 21, 5158-5168.

Zhou, Z. J. and Zhao, D. 2000. Coordinated transitions in neurotransmitter systems for the initiation and propagation of spontaneous retinal waves. *J. Neurosci.* 20, 6570-6577.

Legends

Figure 1: Diagram showing the network connectivity underlying mouse retinal waves at Stage II and Stage III. Stage I waves (mediated by gap junctions) are not illustrated. The top panel shows the retinal network underlying early Stage II waves (late gestation, ~E18, to P3). This network consists of starburst ACs making cholinergic nicotinic connections (nACh) onto RGCs as well amongst each other (indicated by the double headed arrow). The middle panel shows that slightly later in development (from P4), Stage II waves are also controlled by GABA (and glycine to a lesser extent) released by starburst ACs and by other inhibitory ACs. GABA and glycine mediated chloride currents are depolarizing until P6. They switch to their mature inhibitory expression between P6 and P7. The bottom panel shows the network underlying Stage III waves. Lateral cholinergic connections amongst starburst ACs withdraw around P9-10 (indicated by the red cross), and at the same time, glutamatergic connections presumably arising from BC terminals become functional (although it remains possible that the source of glutamate arises also from photoreceptors making transient connections in the IPL or from RGC axon collaterals). At that point, waves are controlled by glutamate and they continue to be modulated by GABA and glycine. Stage III waves completely disappear during the third postnatal week.

Figure 2: Examples of waves recorded in the mouse retina at P3, P5 and P11 with the 4096 channel high density APS MEA. The panels on the left show raster plots of bursts extracted from the recorded spike trains, which were grouped into individual waves (cf. Hennig, M. H. et al, 2009). Each wave is shown in a different color. On the right, three individual waves are plotted (numbers in the left panels indicate the corresponding waves shown). Activity propagation is indicated by a change from bright to dark colors. Grey areas indicate MEA channels where spikes were recorded at some point during the experiment, but that did not participate in the waves shown in the figure. With permission from A. Maccione, M. Gandolfo, M. Hennig, S. Eglén, L. Berdondini and E. Sernagor.

Movie 1: Intrinsic calcium imaging of retinal waves from the GCaMP2 mouse retina (P8). The movie shows two waves recorded from the RGC layer in the same retina. The retina was viewed using a 20x objective. The horizontal span of the image is 500 μm . Images were acquired at 10 Hz. With permission from E. Sernagor, H. Mutoh and T. Knöpfel.

Movie 2: Retinal waves recorded from the mouse RGC layer using the APS high density MEA at P3, P5 and P11. Successive detected waves are represented in different colors for easier separation. The block dots represent all the electrodes that were active at some point during the recording session. The active electrodes cover an area of 2.67x2.67mm. Waves at P3 and P5 are more widespread than at P11. With permission from A. Maccione, M. Gandolfo, M. Hennig, S. Eglén, L. Berdondini and E. Sernagor.