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Report

A Precisely Timed Asynchronous Pattern of ON and OFF Retinal Ganglion Cell Activity during Propagation of Retinal Waves

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SUMMARY

Patterns of coordinated spontaneous activity have been proposed to guide circuit refinement in many parts of the developing nervous system. It is unclear, however, how such patterns, which are thought to indiscriminately synchronize nearby cells, could provide the cues necessary to segregate functionally distinct circuits within overlapping cell populations. Here, we report that glutamatergic retinal waves possess a substructure in the bursting of neighboring retinal ganglion cells with opposite light responses (ON or OFF). Within a wave, cells fire repetitive non-overlapping bursts in a fixed order: ON before OFF. This pattern is absent from cholinergic waves, which precede glutamate-dependent activity, providing a developmental sequence of distinct activity-encoded cues. Asynchronous bursting of ON and OFF retinal ganglion cells depends on inhibition between these parallel pathways. Similar asynchronous activity patterns could arise throughout the nervous system, as inhibition matures and might help to separate connections of functionally distinct subnetworks.

INTRODUCTION

The connectivity patterns of many neuronal networks undergo extensive refinement during development. In particular, many target neurons initially receive exuberant connections from diverse presynaptic cells and with maturation lose inputs from inappropriate synaptic partners while strengthening appropriate connections (Wong and Lichtman, 2003). Molecular cues are thought to govern initial circuit formation, while refinement of synaptic connections appears to be guided by activity-dependent learning rules in many cases (Zhang and Poo, 2001). Since Hebb's original conjecture (Hebb, 1949), many related plasticity rules have been proposed (Dan and Poo, 2006). While the details differ, they commonly predict the strengthening of connections between synchronously active cells and the weakening and elimination of connections between asynchronously active cells. Recently, paired recordings have allowed for direct testing of these rules in mature and developing neural circuits (Dan and Poo, 2006). These studies confirmed the importance of synchronous and asynchronous activity in modifying synaptic efficacy and revealed that the time windows during which pre- and postsynaptic activity interact can vary from millisecond to seconds depending on the dominant activity patterns: spikes and bursts of spikes, respectively (Butts et al., 2007; Sjostrom et al., 2001). In addition, synaptic remodeling in some circuits was shown to be sensitive to the order of pre- and postsynaptic action potentials: strengthening inputs firing before and weakening inputs firing after postsynaptic action potentials (Markram et al., 1997). Critically, in vivo studies that imposed varying activity patterns on retinal ganglion cells (RGCs) converging onto neurons in the tectum of *Xenopus* tadpoles demonstrated that activity-instructed segregation of convergent inputs requires repetitive, precisely timed asynchronous firing of presynaptic cells (Zhang et al., 1998). Although many developing networks are known to spontaneously generate patterns of synchronized activity (Feller, 1999), apart from the partially disjoint firing of motor neurons innervating flexor and extensor muscles in embryonic chick (O'Donovan and Landmesser, 1987), no accurately timed asynchronous activity patterns have been identified.

Retinal waves are the best studied example of spontaneously generated patterned activity (Wong, 1999). In early postnatal development, a network of cholinergic amacrine cells in the inner retina supports the slowly spreading excitation of RGCs correlating their activity in a distance-dependent fashion. Cholinergic waves are required for the normal retinotopic refinement of RGC axons in both the superior colliculus (SC) and dorsolateral geniculate nucleus (dLGN) (Chandrasekaran et al., 2005; Grubb et al., 2003; McLaughlin et al., 2003), as well as for the precise mapping of geniculocortical projections (Cang et al., 2005). Prior to eye opening, when retinal waves are driven by glutamatergic transmission, retinogeniculate projections undergo a further stage of activity-dependent refinement that separates inputs from adjacent ON and OFF RGCs (Dubin et al., 1986), which respond to light increments and decrements, respectively. At the same time, distance-dependent correlations imposed by wave propagation are thought to maintain newly established eye-specific retinotopic maps (Chapman, 2000; Demas et al., 2006), raising the question of whether glutamatergic waves encode cues that help segregate inputs from functionally distinct neighboring cells.

Here, we discovered that the activity of ensembles of neighboring ON and OFF mouse RGCs during glutamatergic waves is precisely coordinated. Within each wave, cell pairs of the same sign display repetitive coincident bursts of action potentials, whereas opposite-signed cell pairs fire adjacent non-overlapping bursts of

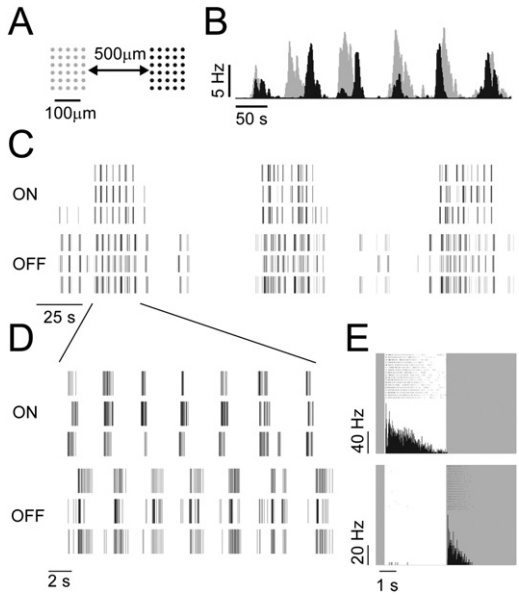

Figure 1. Spontaneous Activity of ON and OFF RGCs during Glutamatergic Waves

(A) Multielectrode array design. Two rectangular recording fields positioned 500 μm apart, each consisting of 30 electrodes (10 μm electrode diameter). The distance between electrodes within each recording field was 30 μm.

(B) Histograms of the average firing rate of all sorted units within a recording field. Color code indicates activity from the respective electrode patches shown in (A).

(C) Spike rasters of spontaneous activity from three ON and three OFF RGCs recorded simultaneously within one recording field. Note that some weaker waves preferentially recruit OFF RGCs.

(D) An excerpt of the spike rasters shown in (C) shown on a finer time scale reveals unique burst structure of ON and OFF RGCs.

(E) Peristimulus rasters and histograms of spike trains from representative ON and OFF RGCs during 18 cycles of a full-field stimulus square wave modulated at 0.125 Hz. Shaded areas indicate periods of darkness ($\sim 10^1$ Rh*/M-cone/s), and unshaded areas indicate periods of illumination ($\sim 10^5$ Rh*/M-cone/s).

action potentials in a fixed temporal order: ON before OFF. We propose that this pattern, which seems to match burst-dependent plasticity rules that guide circuit refinement in the dLGN and SC (Butts et al., 2007; Shah and Crair, 2008), is generated by inhibition between these parallel pathways and a propensity of OFF RGCs to fire upon disinhibition (Margolis and Detwiler, 2007).

RESULTS

To analyze the structure of the spontaneous activity of neighboring ON and OFF RGCs during glutamatergic waves, we recorded spikes at postnatal day 12 (P12) using planar multielectrode arrays as illustrated in Figure 1A. Arrays consisted of two separate recording fields 500 μm apart each containing 30 electrodes within a rectangular area 150 μm by 180 μm. All electrodes of one recording field were contained within an area smaller than the dendritic fields of most RGCs at this age (Diao et al., 2004), allowing us to record the activity of ensembles of neighboring RGCs with overlapping or adjacent dendrites which were recruited nearly simultaneously into passing waves. Comparing the average activity from both recording fields revealed the propagation of activity between both electrode patches that is characteristic of retinal waves (Figure 1B).

To identify ON and OFF RGCs, we presented a square wave modulated (0.125 Hz) full-field stimulus after recording spontaneous activity for >1 hr in complete darkness (Figure 1E). We observed robust light responses in isolated retinas at P12, ~3 days before eye opening. We classified RGCs as ON or OFF responsive if >80% of their spikes occurred within the respective phase of the stimulus and as ON-OFF responsive otherwise. Thus, ~93% of RGCs (519 of 556 RGCs recorded from 39 retinas) were either ON or OFF responsive and ~7% were ON-OFF responsive (37 of 556 RGCs).

Most action potentials of spontaneously active cells at P12 occurred in bursts (81% ± 1%, mean ± SEM) lasting on average 0.61 ± 0.02 s. Similar to recordings of spontaneous glutamatergic activity from ferrets (Lee et al., 2002), we found that OFF RGCs had higher mean firing rates (0.82 ± 0.06 Hz) than ON RGCs (0.48 ± 0.04 Hz, $p < 0.0001$). Interestingly, this was not due to a higher firing rate of OFF compared to ON cells within waves (OFF, 18.1 ± 0.7 Hz; ON, 20.6 ± 1.2 Hz, $p > 0.4$), but to more frequent recruitment of OFF cells into waves of similar length (Figure 1C; $p > 0.1$). Accordingly, the fraction of time spent participating in waves was higher for OFF cells (OFF, 8.1% ± 0.7%; ON, 4.8% ± 0.4%, $p < 0.0001$) and the interval between waves was shorter (OFF, 56 ± 6 s; ON, 127 ± 10 s, $p < 0.0001$).

Neighboring ON and OFF RGCs Fire in Precisely Timed Asynchronous Bursts during Glutamatergic Waves

At P12, RGCs fired multiple bursts of action potentials during a wave. Grouping representative spike trains recorded from the same patch of electrodes according to ON or OFF responsiveness of the cells revealed a striking activity pattern (Figure 1D). Bursts of cells of the same sign appeared to occur together, whereas those of opposite sign did not. In addition, the bursts of ON cells seemed to precede the bursts of OFF cells in stereotypic fashion. To quantify these observations, we computed the crosscorrelations for spike trains of RGC pairs recorded from the same patch of electrodes. Figure 2A shows representative traces for all combinations of cell pairs from one experiment. The crosscorrelation plots confirmed that the burst times of same sign cell pairs were synchronized and those of opposite sign pairs offset such that OFF cells were most likely to fire action potentials ~1 s after the spiking of neighboring ON cells (see also Figure S1 available online). The remarkable precision and stability of this pattern is illustrated by histograms of the peak time in the crosscorrelations of all cell pairs (Figure 2B; 901 pairs, n = 22 retinas). ON and OFF cells thus display precisely coordinated firing patterns during glutamatergic retinal waves, firing adjacent nonoverlapping bursts of action potentials in a fixed temporal sequence: ON before OFF.

Asynchronous Bursting of ON and OFF RGCs Is Restricted to the Period of Glutamatergic Waves

Glutamatergic waves in development are preceded by correlated activity that is supported by a network of cholinergic amacrine cells (Wong, 1999). Cholinergic waves help refine retinotopic

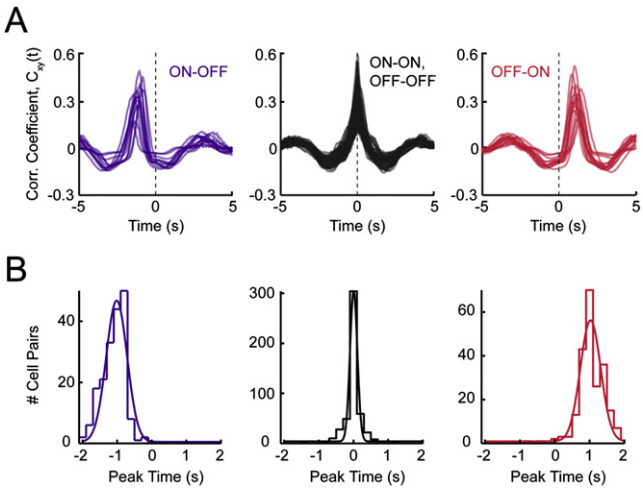

Figure 2. Precise Crosscorrelation Structure of ON and OFF RGC Spiking during Glutamatergic Waves

(A) Crosscorrelations for all combinations of spike trains of spontaneously active RGCs within one electrode field recorded in one experiment at P12. Firing patterns of ON relative to OFF RGCs (left), same-sign RGCs (middle), and OFF relative to ON RGCs (right) are compared.

(B) Histograms representing the peak times in crosscorrelations of all cell-pair combinations (n = 22 retinas) recorded within the same electrode field. Histograms were fit with Gaussian distributions. ON to OFF RGCs (left, mean ± SD, −1.01 ± 0.03 s), same-sign RGCs (middle, 0.01 ± 0.01 s), and OFF to ON RGCs (right, 1.03 ± 0.03 s).

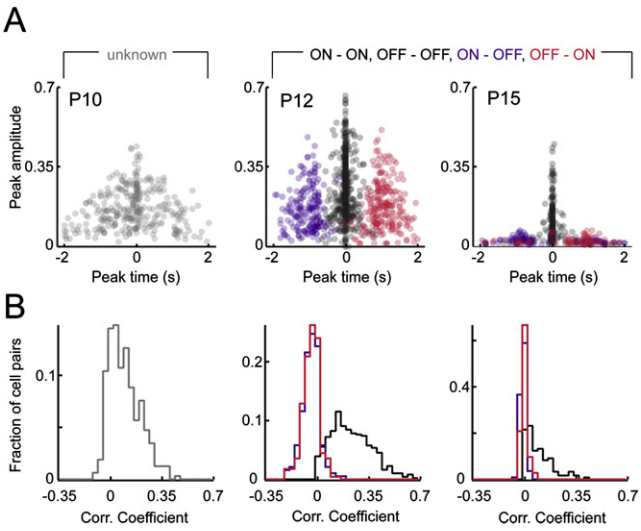

Figure 3. Development of the Crosscorrelation Structure of Spontaneous Activity of ON and OFF RGCs

(A) Each dot represents a pair of cells recorded simultaneously within one recording field at P10 (left, 317 cell pairs, n = 4 retinas), P12 (middle, 901 cell pairs, n = 22 retinas), and P15 (right, 398 cell pairs, n = 5 retinas). x axis values indicate peak time of crosscorrelation functions; y axis values represent the value of the crosscorrelation at its peak. At P10 (left), no reliable light responses were observed, and all cell pairs are therefore depicted as gray dots. At P12 and P15, same-sign cell pairs are shown as black dots, and opposite-sign cell pairs as purple or red dots depending on the orientation of their pairing (purple, ON-OFF; red, OFF-ON).

(B) Histograms of the correlation coefficients (i.e., value of the crosscorrelation function at zero time lag), at P10 (left), P12 (middle), and P15 (right). Color coding as in (A).

maps in subcortical and cortical visual areas but are dispensable for ON/OFF segregation (Cang et al., 2005; Chandrasekaran et al., 2005; Grubb et al., 2003; McLaughlin et al., 2003). We hypothesized that the disjoint burst pattern might be absent from cholinergic waves.

To analyze the development of burst patterns, we compared spontaneous RGC activity at P10 (cholinergic waves), P12 (glutamatergic waves), and P15 (eye-opening, glutamatergic waves in decline) (Bansal et al., 2000; Demas et al., 2003). At P10, we did not observe reliable light responses and thus could not determine if cell pairs consisted of opposite- or same-sign cells. However, when all cell pairs were plotted according to the peak time and amplitude of their crosscorrelation function, they formed a single broad cluster without clear substructure (Figure 3A). Likewise, correlation coefficients were on average positive (0.095 ± 0.006, mean ± SEM, n = 317) and their histogram appeared to outline a single distribution (Figure 3B). To verify this impression, we fit the histogram of correlation coefficients and peak times with Gaussian mixture models with an increasing number of components and calculated Akaike and Bayesian information criteria (McLachlan and Peel, 2000). In both cases, allowing multiple components failed to reduce these criteria for model selection, arguing that activity correlations of nearby RGCs during cholinergic waves were not distinguished by their response type. In contrast, at P12 spike trains from both same- and opposite-sign RGC pairs had high crosscorrelation amplitudes (same, 0.26 ± 0.006, n = 415; opposite, 0.18 ± 0.005, n = 486) with clearly offset peak times, confirming that the activity pattern of ON and OFF RGCs during glutamatergic waves is temporally precise with ON bursts preceding OFF bursts. In addition, correlation coefficients for spike trains were negative for opposite sign pairs and positive for same sign pairs (opposite, −0.045 ± 0.002; same, 0.23 ± 0.007, $p < 0.0001$). Using the same statistical criteria to select the number of components in Gaussian mixture models verified that the histogram of peak times consisted of three populations (ON-OFF, same sign, and OFF-ON pairs) and the histogram of correlation coefficients of two (opposite and same sign). By P15, activity patterns of neighboring opposite-sign RGCs became poorly correlated (correlation coefficient, −0.007 ± 0.002, n = 187, $p < 0.0001$ for comparison to P12) and positive correlation for same sign pairs were reduced (0.097 ± 0.007, n = 211, $p < 0.0001$). This was matched by a decline in waves. While ~80% of spikes occurred during waves both at P10 (85% ± 1%) and at P12 (80% ± 1%), only ~40% of spikes occurred during waves at P15 (40% ± 3%). In conclusion, the precisely timed asynchronous burst pattern of ON and OFF RGCs appears to be restricted to glutamatergic waves, and its expression declines as waves begin to disappear.

Inhibitory Transmission Desynchronizes Burst Times of Neighboring ON and OFF RGCs during Glutamatergic Waves

We performed a series of pharmacological experiments to gain insight into the circuit mechanisms that underlie the distinct burst pattern of ON and OFF RGCs. To facilitate the presentation of

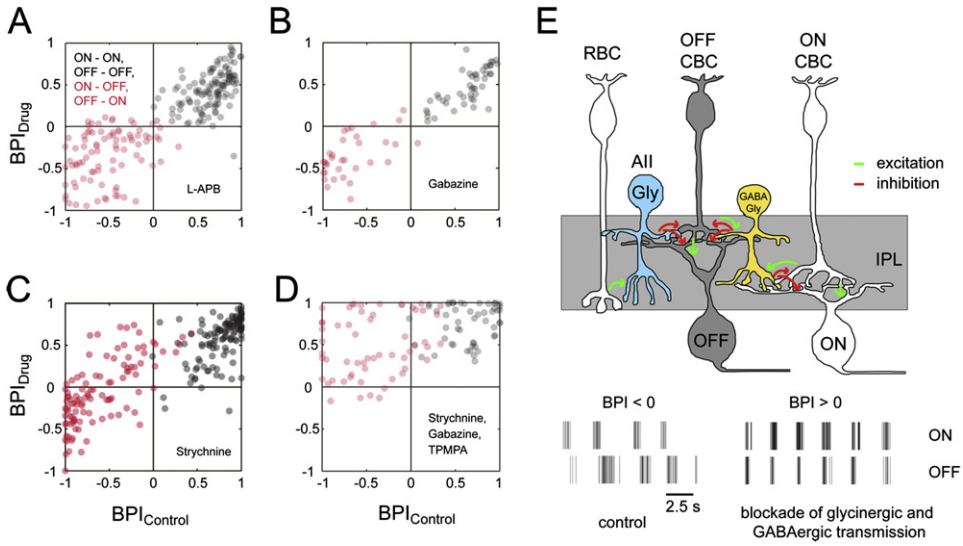

Figure 4. Synaptic Inhibition Underlies Offset Burst Pattern of Spontaneously Active ON and OFF RGCs

(A–D) Burst preference index (BPI) for same-sign (black dot) and opposite-sign (red dot) cell pairs recorded at P12. BPI in control conditions is shown along the x axis. BPI in the presence of the following pharmacological agents is shown along the y axis: (A) 50 μM L-APB (L-2-amino-4-phosphonobutyric acid), (B) 5 μM gabazine (SR95531), (C) 500 nM strychnine, and (D) 50 μM TPMPA, 5 μM gabazine, and 500 nM strychnine. All concentrations of blockers are assumed to be saturating.

(E) Schematic of the mammalian retina. ON circuits are highlighted in white: RBC, rod bipolar cell; ON CBC, ON cone bipolar cell; ON, ON RGC. OFF circuits are highlighted in gray: OFF CBC, OFF cone bipolar cell; OFF, OFF RGCs. A glycinergic (Gly) AII amacrine cell (AII) is shown in blue. The yellow cell represents small-field diffusely stratified amacrine cells that are likely glycinergic (Gly) and mid- to wide-field diffusely or bistratified amacrine cells that are likely GABAergic (GABA) (MacNeil and Masland, 1998; Menger et al., 1998). Red and green arrows indicate the direction of inhibitory and excitatory transmission, respectively, in the inner plexiform layer (IPL). Below the schematic, the burst pattern within one wave in control conditions and during blockade of all inhibitory transmission is shown for representative neighboring ON and OFF RGCs.

pharmacological effects on the crosscorrelation structure of cell pairs, we defined an index of burst preference (BPI, see Experimental Procedures). The BPI is positive for cell pairs that fired more coincident than adjacent bursts and negative for pairs that preferentially fired adjacent bursts.

The mechanisms that shape the defining features of the RGC burst pattern during glutamatergic waves can be addressed by asking two questions: (1) what causes neighboring RGCs of the same sign to burst synchronously, and (2) what causes neighboring ON and OFF RGCs to burst in sequence? For same-sign pairs, blockade of electrical coupling by application of carbenoxolone or meclofenamic acid (Pan et al., 2007) reduced narrow correlations ($\leq$50 ms) but did not change the overall burst pattern (Figure S2). Likewise, blockade of GABAergic (Figure 4B) and/or glycinergic transmission (Figures 4C and 4D) did not affect the burst pattern of same-sign pairs, arguing that their coincident bursting was primarily caused by shared or synchronized excitation from presynaptic bipolar cells. This distinct influence of electrical coupling and bipolar cell input on narrow and broad correlations, respectively, is similar to what was observed previously for correlated activity in the adult retina (Brivanlou et al., 1998).

Interestingly, application of the mGluR6 agonist L-APB (50 μM), which hyperpolarizes ON but not OFF bipolar cells (Slaughter and Miller, 1981), did not affect the relative burst patterns of spontaneously active same- or opposite-sign RGC pairs (Figure 4A). Moreover, L-APB increased the mean rate of spontaneous ON and OFF RGC firing (Table S1), while it blocked all light-evoked ON responses as expected (Figure S3). This indicates that while L-APB-induced hyperpolarization initiated at mGluR6 receptors in ON bipolar cell dendrites is sufficient to block transmission of photoreceptor signals from the outer plexiform layer, it does not block glutamate release from bipolar cell axons during waves in the inner plexiform layer.

Given the circuitry in the inner retina (Figure 4E) and the propensity of OFF RGCs to burst upon disinhibition (Margolis and Detwiler, 2007), the precise sequence in which the bursts of ON cells invariably preceded the bursts of OFF cells suggested that circuits exciting ON RGCs concomitantly suppressed OFF RGCs directly and through inhibition of OFF bipolar cells, followed by postinhibitory rebound firing of OFF RGCs. This hypothesis was tested by blocking GABAergic and glycinergic transmission (Figures 4B–4D). Blockade of $GABA_A$ (gabazine), $GABA_C$ (TPMPA), or glycine receptors (strychnine) increased the average firing rate of RGCs (Table S1) confirming that, as in other species (Fischer et al., 1998; Sernagor et al., 2003), these transmitters are inhibitory during the period of glutamatergic waves in mice. Neither blocking $GABA_A$ (Figure 4B) nor $GABA_C$ (data not shown) receptors alone reversed the burst preference of opposite-sign cell pairs. By contrast, removal of glycinergic inhibition inverted the burst preference of ~30% of opposite-sign pairs toward preferentially firing coincident bursts of action potentials (Figure 4C; 34 of 107, n = 6 retinas, $p < 0.0001$ for reduction of correlation at ±1 s, and increase in correlation at

0 s). Blocking both GABAergic and glycinergic transmission caused reversal of the burst preference for >90% of opposite-sign pairs (Figures 4D and 4E; 53 of 58, n = 5 retinas, $p < 0.0001$ for reduction of correlation at ±1 s, and increase in correlation at 0 s). Together, these results argue that the excitatory drive from ON and OFF bipolar cells to RGCs is indeed synchronized in the absence of inhibitory transmission and that inhibition to OFF RGC exceeds excitation during activity of ON circuits in control conditions. The connectivity of glycinergic AII amacrine cells (Figure 4E) might explain the prominent effect of strychnine which is necessary and in ~30% of the cases sufficient to synchronize the activity of ON and OFF RGCs. When AII amacrine cells are activated through rod bipolar cells during a period of ON circuit activity, they are predicted to further excite ON cone bipolar cells through gap junctions while at the same time both directly and indirectly inhibiting OFF RGCs (Murphy and Rieke, 2008). In addition to this unidirectional ON → OFF inhibitory pathway, several diffusely stratifying glycinergic and GABAergic amacrine cells have been identified (MacNeil and Masland, 1998; Menger et al., 1998), and functional GABAergic crossover inhibition has been described in both directions, albeit with some bias for the ON → OFF orientation (Chen and Linsenmeier, 1989; Roska et al., 2006; Zaghloul et al., 2003). The most cautious explanation of the sequential bursting of ON and OFF RGCs is that ON → OFF inhibition silences OFF RGCs during ON bursts and causes them to burst upon disinhibition at the end of ON bursts (Margolis and Detwiler, 2007).

DISCUSSION

Retinal waves were previously known to synchronize RGC activity in a distance-dependent manner and found to assist in the eye-specific segregation and retinotopic mapping of RGC afferents and geniculocortical projections (Cang et al., 2005; Chandrasekaran et al., 2005; Demas et al., 2006; Grubb et al., 2003; Huberman et al., 2006; McLaughlin et al., 2003). We discovered that glutamatergic waves around eye opening, but not earlier cholinergic waves, comprise a substructure. Within each wave, neighboring ON and OFF RGCs fire a sequence of repetitive asynchronous bursts in a fixed temporal order: ON before OFF. This wave substructure could aid the segregation of neighboring ON and OFF RGC afferents in the dLGN and could potentially help establish early orientation selectivity and ON/OFF domains in the primary visual cortex (V1) (Jin et al., 2008; Miller, 1994). At the same time, distance-dependent correlations imposed by wave propagation likely serve to maintain newly established eye-specific retinotopic maps (Chapman, 2000; Demas et al., 2006).

The activity patterns of like-sign and opposite-sign RGC neighbors match the burst-based plasticity rules recently found to guide synaptic remodeling of retinal projections in developing ferret dLGN (Butts et al., 2007) and mouse SC (Shah and Crair, 2008). Unlike most forms of spike time-dependent plasticity (Dan and Poo, 2006), burst-time-dependent plasticity (BTDP) (Butts et al., 2007) is time symmetric. Coincident bursts of RGCs and dLGN cells strengthen connections between them, whereas adjacent non-overlapping bursts, irrespective of the order in which they occur, weaken and eliminate retinogeniculate synapses. Given some initial bias in the capacity of ON or OFF inputs to drive a particular dLGN cell, their distinct bursting pattern during glutamatergic waves is predicted to drive segregation of opposite-signed convergent inputs.

Since the onset of burst desynchronization between neighboring ON and OFF RGCs coincides with the change from cholinergic to glutamatergic wave propagation (Bansal et al., 2000), it seems that the patterns of cholinergic and glutamatergic waves provide two distinct sets of cues for different aspects of circuit refinement: retinotopy and ON/OFF segregation, respectively. These cues seem to be provided in the burst pattern of RGC firing rather than in individual spikes (Butts and Rokhsar, 2001; Torborg et al., 2005; Figures 1 and 2). Retinotopic refinement and ON/OFF segregation during both periods could thus use common burst-based plasticity rules (Butts et al., 2007; Shah and Crair, 2008). Several lines of evidence support the notion that the developmental sequence, common across many species (Wong, 1999), and balance of cholinergic and glutamatergic waves are crucial for reliable wiring of the visual system. First, bursts of ON and OFF cells during glutamatergic waves are only precisely offset for relatively near RGCs. This activity pattern is therefore expected to most reliably segregate converging ON and OFF afferents in the dLGN after retinotopic refinement. In addition, mice lacking the β2 subunit of nicotinic acetylcholine receptors ($\beta2^{-/-}$ mice), which have no cholinergic waves and early glutamatergic waves, show reduced retinotopic refinement and excessive ON/OFF segregation of retinal afferents (Chandrasekaran et al., 2005; 2007; Grubb and Thompson, 2003; McLaughlin et al., 2003). In the SC, where cells normally respond to both ON and OFF stimuli, in $\beta2^{-/-}$ mice, cells are purely ON or OFF responsive (Chandrasekaran et al., 2007), and in the dLGN, where ON- or OFF-responsive cells are normally intermixed, cells of a given response type form clusters (Grubb et al., 2003). An intriguing question in this context is how the same sequence of retinal activity patterns can lead to the segregation of ON and OFF responses in the dLGN but not the SC in normal development. A possible answer is provided by studies on synaptic remodeling in mice. Chandrasekaran et al. (2007) found that most synaptic remodeling in SC was complete by P7, well before the onset of glutamatergic waves (Bansal et al., 2000). In contrast, Hooks and Chen (2006) observed that in dLGN most synaptic remodeling occurs between P11 and P14, the period spanned by glutamatergic waves (Bansal et al., 2000). Thus, cholinergic and glutamatergic waves appear to provide, in a precise developmental sequence, distinct cues on retinal position and ON or OFF responsiveness. These cues are similarly provided in the burst pattern of spontaneously active RGCs and could instruct circuit refinement by a common burst-based plasticity rule (Butts et al., 2007; Shah and Crair, 2008). The influence of this process in different subcortical target areas appears to be regulated by the timing of distinct critical periods for synaptic remodeling.

We recorded reliable light responses from mouse RGCs in retinal explants during the peak period of glutamatergic waves (P12), around 3 days prior to eye opening. In ferrets, at a comparable stage of development, dLGN cells were shown to respond to naturalistic visual stimuli presented through closed eyelids (Akerman et al., 2002), raising the possibility that early visual activity might instruct ON/OFF segregation in the dLGN. Accordingly, dark rearing prior to eye opening led to an increased

convergence of ON and OFF responses (Akerman et al., 2002). By contrast, in mice, during the period of glutamatergic waves, blockade of spontaneous activity but not visual deprivation was found to delay synaptic remodeling and pruning of RGC inputs to dLGN cells (Hooks and Chen, 2006). This suggests that spontaneous, rather than visually evoked, activity is dominant in guiding normal maturation of RGC projections in mice. Note that both the spontaneous activity patterns we discovered here and early visual responses desynchronize the activity of neighboring ON and OFF RGCs. Thus, spontaneous and visually evoked activity could instruct ON/OFF segregation together. The apparently different relative importance of these sources of activity for ON/OFF segregation in mouse and ferret dLGN could in part be due to the different structure of RGC activity during glutamatergic waves (Lee et al., 2002; Figure S4).

In addition to shaping connectivity patterns of RGC axons in subcortical visual areas, retinal waves, which can drive bursting of cells in dLGN and V1 (Hanganu et al., 2006; Mooney et al., 1996), have been proposed to influence several aspects of geniculocortical mapping including retinotopy (Cang et al., 2005), formation of ocular dominance columns, and the size of binocular receptive fields in V1 (Huberman et al., 2006). Evidence for the influence of spontaneous retinal activity on cortical wiring is thus far limited to the period of cholinergic waves. Interestingly, however, models of activity-driven development of orientation selectivity in V1 require activity patterns that desynchronize the firing of neighboring cells of opposite sign while at larger retinotopic separations ON and OFF cells should be coactive more often than cells of the same sign (Miller, 1994). The substructure of glutamatergic retinal waves appears to fit these requirements. Further, if the RGC burst pattern during glutamatergic waves is faithfully transmitted to dLGN cells, it might help establish ON and OFF domains of dLGN afferents in V1 (Jin et al., 2008).

An interesting parallel to the desynchronization of bursts of ON and OFF RGCs which accompanies the transition from cholinergic to glutamatergic waves is the partially disjoint spontaneous firing of extensor and flexor motorneurons that gradually develops in chick embryos (O'Donovan and Landmesser, 1987). Taken together, these findings raise the possibility that spontaneous activity patterns generated by many early neural circuits undergo similar changes as connectivity matures; displaying precisely timed asynchronous activity that could help segregate functionally distinct subnetworks throughout the nervous system.

EXPERIMENTAL PROCEDURES

Recordings

Multielectrode array (HD30/10-ITO-pr, Multi Channel Systems, Reutlingen, Germany) recordings of retinas from C57Bl/J6 mice were performed as described previously (Demas et al., 2003). A monochromatic yellow organic light-emitting display (OLED, eMagin, Bellevue, WA) was used to present a full-field stimulus square wave modulated at 0.125 Hz. Stimulus intensity was equivalent to $\sim 10^5$ photoisomerizations per middle-wavelength sensitive cone per second (Rh*/M-cone/s) during ON phase of the stimulus and $\sim 10^1$ Rh*/M-cone/s during its OFF phase.

Data Analysis

Data were analyzed using custom software written in Matlab (Mathworks, Natick, MA). We calculated the crosscorrelation for spike trains of cell pairs in the same recording field according to:

$$C_{xy}(t) = \begin{cases} \dfrac{\frac{1}{N-\frac{t}{\Delta t}} \times \sum_{i=1}^{N-\frac{t}{\Delta t}} (x_i - \langle x \rangle) \times \left(y_{i+\frac{t}{\Delta t}} - \langle y \rangle\right)}{\sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \langle x \rangle)^2} \times \sqrt{\frac{1}{N}\sum_{i=1}^{N}(y_i - \langle y \rangle)^2}} & t \geq 0 \\ C_{yx}(-t) & t < 0 \end{cases} \quad (1)$$

where x_i and y_i represent spike counts of two cells in the i-th of N time bins, $< x >$ and $< y >$ signify their respective average spike counts, and t the time lag in the crosscorrelation. The width of time bins (Δt) for the results shown was 50 ms. To verify that our conclusions did not depend on the choice of bin width, we analyzed crosscorrelation with a range values (10 ms–500 ms) obtaining qualitatively similar results. Because the firing rate of spontaneously active RGCs was nonstationary at the ages recorded (relatively high during waves and negligible in between), we determined average spike counts using a 5 s-wide sliding window (Perkel et al., 1967). This effectively removed the positive correlation otherwise added for cells that are both silent between waves. We systematically varied the width of the averaging window between 2 s and 20 s to verify that our conclusions were independent of the precise choice of this parameter. Due to the length of spike trains used in this study (~1 hr recordings divided into 50 ms time bins) threshold for statistical significance of correlation coefficients ($C_{xy}(0)$) were below $|C_{xy}(0)| = 0.01$.

To demonstrate effects of pharmacological agents on the cross-correlation structure of cells within the same recording field, we defined an index of burst preference (BPI) as

$$BPI = \frac{R_{xy}(0s) - \frac{1}{2}(R_{xy}(1s) + R_{xy}(-1s))}{R_{xy}(0s) + \frac{1}{2}(R_{xy}(1s) + R_{xy}(-1s))} \quad (2)$$

where $R_{xy}(t)$ is the raw cross correlation of two cells at lag t:

$$R_{xy}(t) = \begin{cases} \dfrac{1}{N - \frac{t}{\Delta t}} \sum_{i=1}^{N-\frac{t}{\Delta t}} x_i \times y_{i+\frac{t}{\Delta t}} & t \geq 0 \\ R_{yx}(-t) & t < 0 \end{cases}. \quad (3)$$

Variables in Equation 3 have the same meaning as in Equation 1. The BPI uses the crosscorrelation at zero time lag to measure the likelihood of two cells (x and y) to fire coincident bursts and the average correlation at −1 s and 1 s to assess the likelihood of cell x to burst before or after cell y, respectively. The use of these time lags was based on the position of the peak in the crosscorrelation of opposite-sign cell pairs (Figures 2 and 3) and the average burst width of RGCs in our experiments (0.61 ± 0.02 s at P12). The BPI is expected to be positive for cell pairs that fire more coincident than adjacent bursts and negative for those that preferentially fire adjacent bursts. We used the raw crosscorrelation to ensure that the BPI was bound between 1 and −1.

Throughout this study we used either Wilcoxon-Mann-Whitney rank sum or, in case of paired samples, Wilcoxon signed-rank tests to assess statistical significance of differences between groups.

Additional details of recordings and data analysis are described in the Supplemental Experimental Procedures.

SUPPLEMENTAL DATA

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/58/6/851/DC1/.

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